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Red Knot diet reconstruction revisited: context dependence revealed by experiments at Banc d'Arguin, Mauritania

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Capsule Context-specific equations are needed to reconstruct diet composition and intake rate of Red Knots by the use of shell fragments retrieved from droppings.

Aims To explore whether the method to reconstruct Red Knot diet described by Dekinga & Piersma [Dekinga, A. & Piersma, T. 1993. Reconstructing diet composition on the basis of faeces in a mollusc-eating wader, the Knot *Calidris canutus*. *Bird Study* 40: 144–156] accurately predicts the diet of Red Knots *Calidris canutus canutus* outside Northwest Europe, at Banc d'Arguin, Mauritania.

Methods Feeding experiments with captive Red Knots on the bivalves *Dosinia isocardia* or *Loripes lucinalis* were carried out at Banc d'Arguin, the main wintering area of Red Knot subspecies C. *c. canutus*. Ingested diets were compared with the reconstructed diets derived from the general method developed by Dekinga & Piersma (1993). Droppings collected over multiple years were also analysed to evaluate the calibration method from this study.

Results Of the total ingested shell mass (DM_{shell}) in both bivalves, approximately 65% of the shell mass was retrieved in the droppings (DM_{drop}). Therefore, dry mass of droppings in the field (DM_{drop}) has to be multiplied by 1.547 to calculate the ingested dry mass (DM_{shell}). For size estimations of ingested shells from droppings, hinges should be used for *D. isocardia* and hinges including tops for *L. lucinalis*.

Conclusion The correction factor of 1.547 found here is 50% larger than the factor 0.993 for heterodont bivalves from Europe established by Dekinga & Piersma (1993). Application of the published factor would lead to serious underestimation of energy intake rates based on dropping frequencies and dropping content (by as much as 35%), although it would have small effects on the relative species composition of the diet. Having shown that such correction factors can differ among sites and prey we recommend their determination in new ecological contexts.

The diet of an animal is of wide interest, because it reveals the position of a consumer in a food web (Sheppard & Harwood 2005). One way to estimate diet composition is to reconstruct it from faeces, a method that is widely applied to a variety of animals, especially when the studied animals are endangered or vulnerable to disturbance (Price *et al.* 2005, McFadden *et al.* 2006), when their prey are hard to identify by remote observations (Scheiffarth 2001, Lee & Severinghaus 2004), or when the animals are just too elusive to directly observe their feeding behaviour (Chame 2003, Sheppard & Harwood 2005). When recognizable prey fragments can be found in faeces, their relative abundance in the faeces may reflect what is actually consumed (Putman 1984, Dekinga & Piersma 1993, Hammill *et al.* 2005). However, digestion processes can alter prey structures to such an extent that faecal analysis can give a biased reconstruction of the diet (Jenni *et al.* 1990, Bowen 2000, Jarman *et al.* 2002). Calibration studies can correct for such estimation problems (Dekinga & Piersma 1993). Nowadays, modern techniques like genetic markers and stable isotope analysis can help to overcome part of these problems (Kohn & Wayne

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1997, Fedriani & Kohn 2001, Bradley *et al.* 2007, Oehm *et al.* 2011). However, these techniques are even more time consuming and more expensive than the relatively simple analysis of hard parts of prey retrieved from faeces (Barrett *et al.* 2007).

One of the better studied bird species in terms of their diet in a wide range of field contexts is the Red Knot (*Calidris canutus*) (Piersma & van Gils 2011, Piersma 2012). It can be observed and counted with ease while foraging in open and accessible habitats. Being molluscivore specialists, Red Knots migrate from their tundra breeding grounds along intertidal soft sediment habitats where molluscs are available (Piersma 2007). These hard-shelled prey are swallowed whole and crushed in their muscular gizzard (Piersma *et al.* 1993). This large organ is rapidly adjusted in size when there are shifts in the hardness of the food ingested (Dekinga *et al.* 2001, van Gils *et al.* 2006). As maintaining a large gizzard is energetically costly, prey with a high flesh-to-shell ratio are preferred by energy maximizing Red Knots (van Gils *et al.* 2005b).

The intertidal mudflats of Banc d'Arguin, Mauritania, northwest Africa, are the main wintering sites of Red Knot subspecies *Calidris canutus canutus* (Piersma & Davidson 1992, Leyrer *et al.* 2012). Here, two bivalve species *Loripes lucinalis* (Mollusca, Bivalvia, hereafter *Loripes*) and *Dosinia isocardia* (Mollusca, Bivalvia, hereafter *Dosinia*) are numerically the most abundant prey species, making up 69% of all molluscs (Honkoop *et al.* 2008), and form the main species in the diet of Red Knots at Banc d'Arguin (van Gils *et al.* 2012, 2013).

Van den Hout (2010) reconstructed the diet of Red Knots in Banc d'Arguin by estimating ingested shell mass from sieved dropping mass using the equations of Dekinga & Piersma (1993), who carried out a calibration study for Red Knots feeding in northwest Europe. Although Dekinga & Piersma (1993) stated that these equations would likely be globally applicable for heterodont bivalves, this remained to be tested. Shell thickness and resistance to crushing vary among species of molluscs (Cabral & Jorge 2007) and even among seasons (Nagarajan *et al.* 2006). As *Dosinia* and especially *Loripes* are thin-shelled prey that can be crushed easily (Yang *et al.* 2013), we were concerned that a large fraction of shell mass in droppings is lost in the sieving process given the fixed mesh of 300-µm used by Dekinga & Piersma (1993). We expect this fraction to be larger than the fractions found by Dekinga & Piersma (1993) for more thick-shelled prey from the Wadden Sea.

This methodological study aims to reconstruct the diet of Red Knots in Banc d'Arguin by using the method outlined by Dekinga & Piersma (1993), but calibrated for Banc d'Arguin prey items. This calibration study consists of three steps:

(1) Calculation of DM_{drop}/DM_{shell} ratio to arrive at the correction factor for calculating the total ingested sieved dry shell mass (DM_{shell}) from dry sieved shell mass of the droppings (DM_{drop}).
(2) Reconstruction of the ingested shell size distributions.
(3) Calculation of α, the species-specific flesh-to-shell mass ratio, which is needed for the calculation of diet composition in terms of ingested biomass.

Captive Red Knots were given either *Loripes* or *Dosinia*. The offered and left-over prey were used to determine the ingested diet, which was compared with the excreted amount of shell fragments in the droppings. Diet reconstructions on the basis of the newly derived factors were then compared with diets reconstructed from the equations presented by Dekinga & Piersma (1993). For an evaluation of the implications of our study, droppings collected in the field in three consecutive years (2007–2009) in Banc d'Arguin were used for diet reconstruction, and our results were compared with the results obtained by using the method of Dekinga & Piersma (1993).

METHODS

Experiments: Banc d'Arguin, Mauritania

The study was conducted at the Iwik field station (19° 52.42′ N, 16°18.50′W) in Parc National du Banc d'Arguin, Mauritania, between 10 January and 2 February 2011. Six Red Knots were caught with mist nets near a roosting site approximately 2.5 km from the field station. The flock consisted of three second calendar-year birds and three >second calendar-year birds (based on their plumage). Average bill length of these birds was 34.6 mm (range 30.3–38.0 mm) and average body mass just after catching was 115.5 g (range 96–127 g). Every morning each bird was weighed and its health status assessed. Daily food supply was adjusted to keep the body mass just above the lean body mass of ca. 100 g for the birds to keep their motivation to feed during the experiments. Between experimental trials, the birds were kept together in a small indoor aviary (150 cm × 100 cm

× 50 cm) with a layer of beach sand on the ground and *ad libitum* fresh water. Staple food consisted of high-quality unshelled *Senilia senilis* flesh pieces (a large and very common bivalve in Banc d'Arguin) and commercial trout feed (*TrouVit*; Produits Trouw, Vervins, France).

A total of 48 trials were carried out: 24 with *Dosinia*-diet and 24 with *Loripes*-diet that were equally divided over six birds and over 12 days. During daytime, the birds were placed in an experimental unit (150 cm × 100 cm × 50 cm) that was subdivided with transparent panels into six compartments of 50 cm × 50 cm, each of which held a single bird. To prevent droppings being trampled by the birds, the ground surface consisted of plasticized wire mesh (10 mm × 10 mm) placed 1 cm above the bottom of the experimental unit. Droppings fell through the mesh and were collected after the trial and stored in the freezer per trial before analysis in the laboratory at NIOZ, the Netherlands. During the night before the trials only shellfish flesh was offered (no shelled prey items) to be sure that no fragments of former diet remained in the digestive tract: droppings produced just prior to the trials did not contain shell fragments. Furthermore, to ensure that the birds ate eagerly during the trials, no staple food was offered for at least 6 hours before the start of the trial. In each trial, a single prey type was offered unburied and *ad libitum*, covering the ingestible size spectrum. After 6 hours all leftover prey items were removed and the birds had 4 hours to empty their guts, which ensured that all shell materials were excreted (following Dekinga & Piersma 1993).

Dosinia was collected at the beach just east of the field station and *Loripes* at the sea grass beds of Abelgh Eiznaya (2 km NW from field station). Both species were collected with a sieve (2-mm mesh) on the day before the trials and kept overnight in the refrigerator to prevent loss of body mass and to keep them in good condition. To reconstruct the consumed size distributions, samples of prey offered and leftover were taken.

Laboratory analysis

Dekinga & Piersma (1993) emphasized that a calibration for measurable prey fragments in faeces that are allometrically related to prey size and prey size–biomass relationships still has to be made for other prey species at other locations. Shell lengths of ingested molluscs can be reconstructed from measurable fragments such as hinges for bivalves and the width of the last whorl for snails. To reconstruct shell length by the use of hinges (the parts of a shell where the two valves are joined) or hinge plus tops (a swelling above the hinge line, also called umbo), 105 *Dosinia* and 106 *Loripes* shells were used. Fig. 2 gives an illustration of hinges and hinge plus tops of *Dosinia* and *Loripes*. Hinges and hinges plus tops of both valves were measured, and the average of both valves was used for the calibration curves to avoid pseudoreplication. Calibration curves were constructed for *Dosinia* and *Loripes* based on a new collection of 125 *Dosinia* specimens and 123 *Loripes* specimens collected across the experimental period. These were used also to determine the following characteristics: shell length, dry shell mass (DM_{shell}, after drying to constant mass for 3 days at 55–60°C) and ash-free dry mass of the flesh ($AFDM_{flesh}$, after incinerating the dry flesh material overnight at 550°C).

Droppings (for collection details, see below) were washed and sieved over a 300-μm mesh (standard mesh size for dropping analyses in the field to remove sand and dirt; Dekinga & Piersma 1993). Residue (hereafter called dry mass of droppings; DM_{drop}) and filtrate were collected and after drying to constant mass for 3 days at 55–60°C, both the fractions were weighed to the nearest 0.1 mg. A subsample of DM_{drop} was weighed and all hinges were counted in order to calculate the percentage of hinges retrieved in the droppings. Fifty measurable parts were randomly collected and measured by the same observer (J.O.), using a binocular (Olympus SZ51) with eye-piece micrometer (WHSZ 10×-H/22).

The size distribution of the offered and leftover (uneaten) prey was calculated by taking a random sample (range of sample sizes: 54–140 and 4–156 shells, respectively) of the total supply offered and leftover prey of which we measured shell length with callipers to nearest 0.01 mm. The subtraction of the leftover size distributions from the offered distribution provided an estimation of the number of prey items consumed per mm size class based on real prey sizes. Subsequently, calibration curves for shell length (SL) against dry shell mass provided the dry shell masses. For equations, see Table 1. Diet composition in terms of ingested biomass (i.e. ash-free dry mass of flesh $AFDM_{flesh}$) can be calculated from the total ingested dry shell mass by multiplying DM_{shell} with α, the species-specific flesh-to-shell mass ratio. However, flesh-to-shell mass ratios are size-dependent and taking into account the size distribution to calculate α generally improves the estimated flesh mass (Dekinga

Table 1. To convert shell length (SL, mm) into ash-free dry mass of the flesh ($AFDM_{flesh}$, g) and dry mass of the shell (DM_{shell}, g), the equations listed in this table were used.

Species	Linear regression	R^2	*P*-value
Loripes	$AFDM_{flesh} = 1.61E{-}05*SL^{2.977}$	0.95	<0.001
	$DM_{shell} = 6.38E{-}05*SL^{3.250}$	0.96	<0.001
Dosinia	$AFDM_{flesh} = 1.48E{-}05*SL^{2.777}$	0.95	<0.001
	$DM_{shell} = 3.69E{-}05*SL^{2.625}$	0.96	<0.001

Note: Data were obtained from specimens collected during the experimental period between 10 January and 2 February 2011.

& Piersma 1993). Therefore, we calculated α per length class and used the weighted mean α across all length classes taking into account the relative frequency of each length class in a trial.

Reconstructing diet from field droppings

The procedural steps and equations needed to quantify *Dosinia* and *Loripes* in the diet of Red Knots are listed in Table 2. A total of 51 dropping samples (representing a total of 2179 droppings; mean = 60.5 droppings per sample, sd = 46.0) of Red Knots in Banc d'Arguin were collected at seven tidal flats distributed around the Iwik peninsula (a 50 km^2 subsection of Parc National du Banc d' Arguin) during four expeditions (spring 2007, autumn 2007, winter 2007/2008 and autumn 2009). Droppings were stored, dried and sorted out as outlined above. To evaluate the significance of our study, diet reconstruction of these samples was done by two different methodologies: (1) the outcome of this study and (2) based on Dekinga & Piersma (1993). As no correction factors were calculated for other species than *Loripes* and *Dosinia*, the equations by Dekinga & Piersma (1993) were used for gastropods and other bivalves (with correction factors of 1.267 and 0.994, respectively) to arrive at DM_{shell} in the first methodology (obviously, in the second methodology we used the correction factor 0.994 also for *Loripes* and *Dosinia*).

Furthermore, we used these 51 dropping samples to calculate the energy value per dropping. In avian diet studies, this value is multiplied with the dropping rate to estimate the energy intake rate (Bedard & Gauthier 1986, Dekinga & Piersma 1993, Piersma *et al.* 1994, González *et al.* 1996). Hence, any estimation error in the energy value per dropping carries through in the calculation of energy intake rate.

Statistical analyses were performed using *R* (R Development Core Team 2011), and graphs were produced using SIGMAPLOT 12.0 for Windows (Systat Software Inc. 2010).

Table 2. Summary of the procedures to arrive at an estimate of diet composition on the basis of faeces from Red Knots feeding on *Loripes lucinalis* and/or *Dosinia isocardia*.

Setting	Procedure	Measured/estimated parameters
Field	Collect a number of droppings in an area where Red Knots were foraging for 45 min or longer	n_{drop}
	Store droppings dry or frozen	
Laboratory	Dry the droppings to constant mass at 55–60°C	
	Sort over 300-μm sieve to remove sand, dirt and smallest fragments	
	Weigh the fraction retained on the sieve	DM_{drop}
	Manually sort the sieved fraction in fragments from different prey species under binocular and weigh	Partial DM_{drop}
	Measure identifiable fragments with ocular micrometer	Hinge (H;mm) and Hinge plus top (HT; mm)
Desk	Compute prey size (distribution) and diet parameters	
	Loripes	
	$SL = 10.002*HT^{0.950}$	Shell length (SL; mm)
	$DM_{shell} = 1.547\ DM_{drop}$	Shell mass (DM_{shell})
	$AFDM_{flesh} = DM_{shell}$*mean $AFDM_{flesh}/DM_{shell}$ ratio (α)	Flesh mass ($AFDM_{flesh}$)
	Dosinia	
	$SL = 10.254*H^{0.872}$	Shell length (SL; mm)
	$DM_{shell} = 1.547\ DM_{drop}$	Shell mass (DM_{shell})
	$AFDM_{flesh} = DM_{shell}$*mean $AFDM_{flesh}/DM_{shell}$ ratio (α)	Flesh mass ($AFDM_{flesh}$)

Note: These equations are applicable for droppings collected in Banc d'Arguin, Mauritania.

RESULTS

Reconstructing ingested dry shell mass from droppings

For both bivalve species, a significant fraction of the total ingested dry shell mass was lost through the 300-μm mesh (Fig. 1). A linear model showed no effect of species on intercept or slope of the regressions of DM_{shell} on DM_{drop} ($t = -1.826$, $P = 0.07$, df = 45). Furthermore, the model without intercept was more parsimonious ($R^2 = 0.97$, $P < 0.001$, df = 47, AIC = 289.51) than the model with intercept ($R^2 = 0.90$, $P < 0.001$, df = 46, AIC = 289.91). We therefore estimated

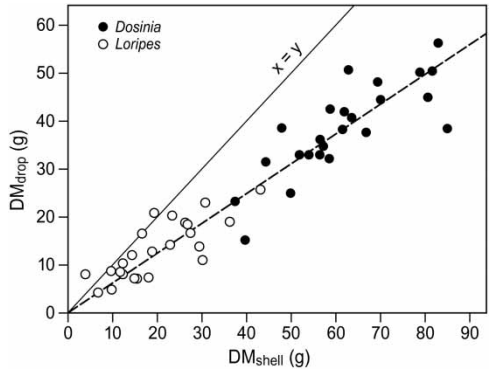

Figure 1. Dry mass of droppings retained on 300-µm sieve (DM_{drop}) plotted against ingested dry shell mass (DM_{shell}) for *Dosinia isocardia* (filled dots) and *Loripes lucinalis* (open dots). Each data point stands for a single trial. The regression for $DM_{drop} = 0.646\ DM_{shell}$ ($R^2 = 0.97$, $P < 0.001$, df = 47, dashed line) applies to both prey species.

the ingested dry shell mass from the dropping mass retained on a 300-µm mesh by the equation: $DM_{shell} = 1.547\ DM_{drop}$ ($R^2 = 0.97$, $P < 0.001$, df = 47), meaning that 65% of the shell mass was retained for both species (Fig. 1).

Reconstructing size distribution by the use of hinges

Reconstruction of the size classes ingested by Red Knots feeding on either *Loripes* or *Dosinia* is based on the allometric relationship between heights of the hinge (H) or based on the height of the hinge plus top (HT) and shell length (SL) of these bivalves. For *Loripes*, measuring hinge and top together provided a slightly more reliable estimation of the shell length than measuring hinge (H) alone (Fig. 2; $SL = 10.002\ HT^{0.950}$, $R^2 = 0.84$, $P < 0.001$, df = 105, AIC = −383.37 and $SL = 14.807\ H^{0.811}$, $R^2 = 0.79$, $P < 0.001$, df = 105, AIC = −354.67). Although for *Dosinia*, shell length was best estimated by measuring hinge plus top (Fig. 2; $SL = 7.145\ HT^{0.891}$, $R^2 = 0.90$, $P < 0.001$, df = 103, AIC = −391.421 and $SL = 10.254\ H^{0.872}$, $R^2 = 0.87$, $P < 0.001$, df = 103, AIC = −359.56), we found that using hinges only provided a more reliable estimation of average reconstructed shell lengths (Fig. 3) and reconstructed size distributions (Fig. 4). Therefore, we recommend using hinge plus tops for Loripes and using hinges only for Dosinia, as we do throughout this paper. On average, 24% (sd = 7%, range = 11–38%) and 21% (sd = 4%, range = 14–29%) of the measurable hinges were found intact in the droppings for *Dosinia* and *Loripes*, respectively. Average ingested shell length per trial was correctly predicted by average reconstructed shell length for both bivalve species when using the correct species-specific measurable shell fragment (Fig. 3). Based on hinges (*Dosinia*) or hinge plus tops (*Loripes*) from faeces, the above-mentioned regression equations were used to predict the size distributions, which were compared with the actual size distribution being eaten

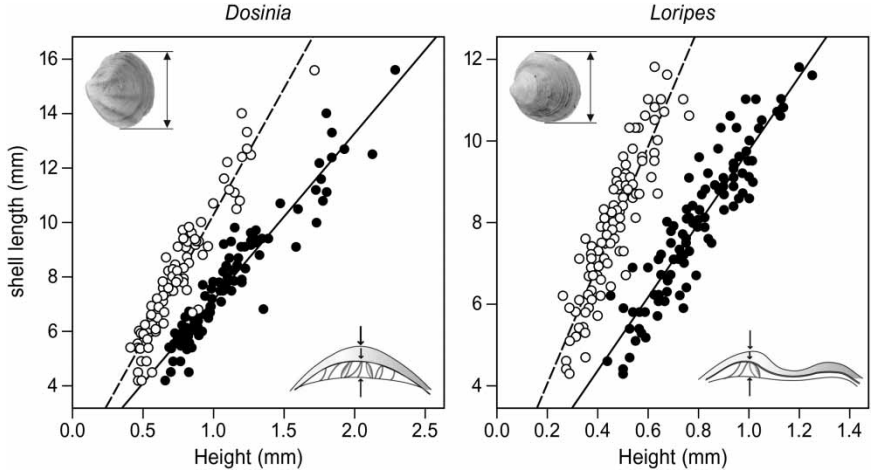

Figure 2. Shell length (SL; mm, measurement method is shown in top left inset) from two bivalve species in Banc d'Arguin, Mauritania, *Dosinia isocardia* (left panel) and *Loripes lucinalis* (right panel) as a function of hinge (H; mm; open dots) and hinge plus top (HT; mm; filled dots) height. The inset in the bottom right corner represents a cross-section of the bivalves' measurable fragments with the outer arrows emphasizing the hinge plus top and the inner arrows only the hinge. The double-logarithmic regression line shown is represented by the following equations: *Loripes*: $SL = 10.002*HT^{0.950}$ ($R^2 = 0.84$, $p < 0.001$, $n = 106$) and $SL = 14.807*H^{0.811}$ ($R^2 = 0.79$, $p < 0.001$, $n = 106$) and for *Dosinia*: $SL = 7.145*HT^{0.891}$ ($R^2 = 0.90$, $p < 0.001$, $n = 105$) and $SL = 10.254*H^{0.872}$ ($R^2 = 0.87$, $p < 0.001$, $n = 105$).

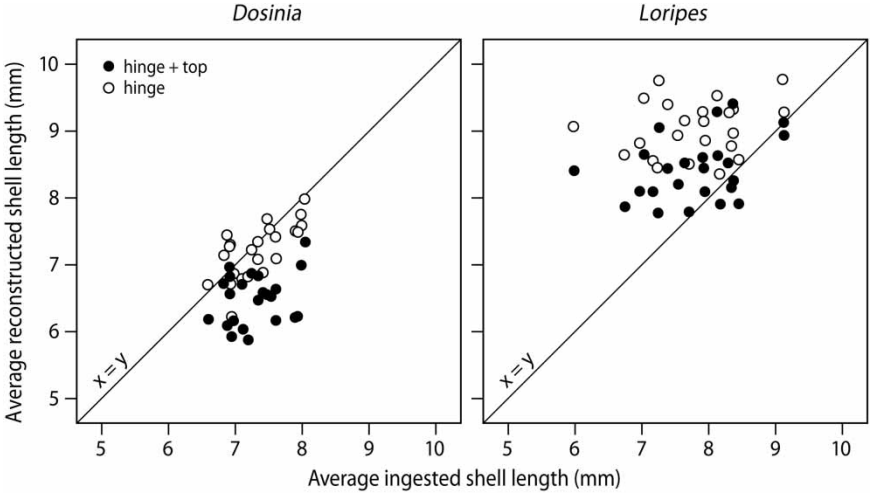

Figure 3. Average ingested shell length as the function of average reconstructed shell length for *Dosinia isocardia* (left panel) and *Loripes lucinalis* (right panel) calculated by using hinges (open dots, for *Dosinia*: $R^2 = 0.45$, $p < 0.001$, df = 22 and for *Loripes*: $R^2 = 0.05$, $p = .30$, df = 22) or using hinges plus tops (filled dots, for *Dosinia*: $R^2 = 0.07$, $p = 0.11$, df = 22 and for *Loripes*: $R^2 = 0.12$, $p = 0.09$, df = 22). Each dot denotes a single trial.

(Fig. 4). A Kolmogorov–Smirnov test did not show a significant difference between these distributions, for *Dosinia*: $D = 0.22$, $P = .77$ and for *Loripes:* $D = 0.25$, $P = .85$.

Estimating diet with respect to biomass

For both *Dosinia* and *Loripes*, there was a nonlinear relationship between α and shell length (Fig. 5). Therefore, α has to be calculated per shell length class (i.e. length-specific $AFDM_{flesh}$ divided by length-specific DM_{shell}) before calculating a species' average α (see Table 1 for equations).

Diet reconstruction of Red Knots in Banc d'Arguin

Relative contributions to ingested $AFDM_{flesh}$ were underestimated by 3% for *Dosinia* and *Loripes* when using the calibration factors of Dekinga & Piersma (1993) (Fig. 6a). Although this is just a small underestimation, the difference is as large as 35% when the energy value per dropping is calculated (Fig. 6b).

DISCUSSION

This study outlines the newly fitted equations to estimate diet composition, prey-size selection and energy intake rate based on droppings of Red Knots

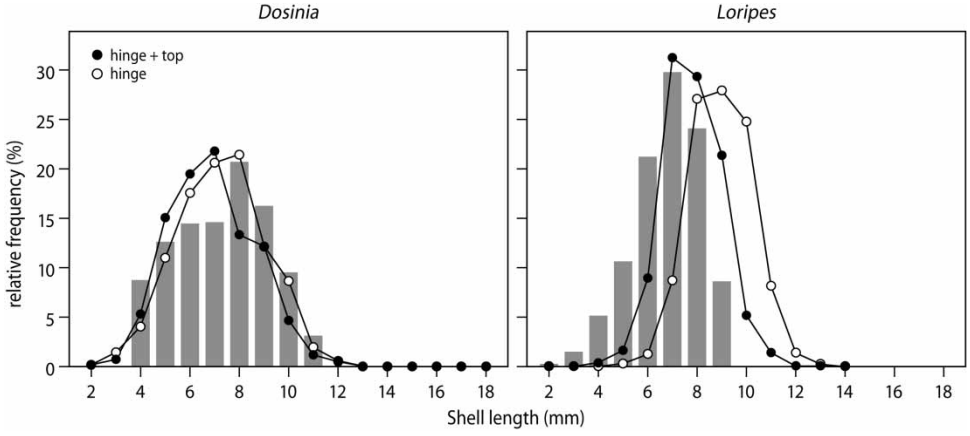

Figure 4. Shell length distributions of *Dosinia isocardia* (left panel) and *Loripes lucinalis* (right panel) ingested by Red Knots (histograms) and the shell length distribution reconstructed on the basis of heights of hinges plus tops (lines with filled dots) and hinges (lines with open dots) of *Dosinia isocardia* and *Loripes lucinalis,* respectively, retrieved from their droppings. For each prey species, the plots represent the cumulative data of 24 trials.

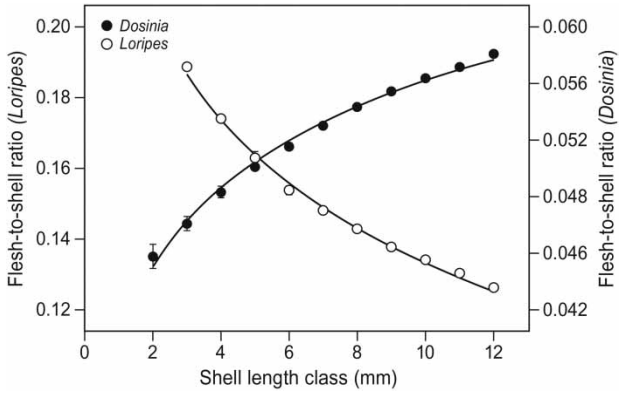

Figure 5. Flesh-to-shell ratios (α) per length class with error bars (se) for the two bivalve species *Dosinia isocardia* (filled dots) and *Loripes lucinalis* (open dots). Nonlinear regression lines are plotted for the data (for *Dosinia*: 0.040 + 0.007 ln(SL), $R^2 = 0.99$, $p < 0.001$, df = 9 and for *Loripes*: 0.236–0.045 ln(SL), $R^2 = 0.99$, $p < 0.001$, df = 9).

feeding on *Loripes* or *Dosinia* in Banc d'Arguin. For both bivalve prey species about 65% of the total ingested shell dry mass (DM_{shell}) is retrieved in dry mass of the droppings (DM_{drop}) after sieving. Thus, to calculate the ingested DM_{shell} for true diet compositions, DM_{drop} has to be multiplied by 1.547, rather than by 0.994 found by Dekinga & Piersma (1993) and claimed to be globally applicable for heterodont bivalves. Previous studies that reconstructed Red Knot's diet in Banc d'Arguin on the basis of the 0.994 factor (van den Hout 2010) slightly underestimated the contribution of *Dosinia* and *Loripes* to the diet (Fig. 6a). Furthermore, future studies that would use the 0.994 factor would underestimate energy intake rate from defecation rates by about 35% (Fig. 6b).

The use of hinges only for *Dosinia* and hinges plus tops for *Loripes* in the method described by Dekinga & Piersma (1993) provides a good estimation to reconstruct the size distributions of Red Knot diet (Fig. 4), in spite of the fact that only a small fraction (24% and 21% for *Dosinia* and *Loripes*, respectively) of the total ingested hinges was relocated. Dekinga & Piersma (1993) similarly found a low percentage of hinges in droppings produced after Red Knots ate two species of Wadden Sea bivalves (45% for *Macoma balthica* and 11–14% for *Cerastoderma edule*). Furthermore, just as in the bivalves studied by Dekinga & Piersma (1993), no hinges of particular size classes were lost more than other size classes, resulting in an unbiased reconstructed size distribution (Fig. 4).

The size of the gizzard might influence the crushing capacity of the ingested shell material and thus the measurable fragments in the faeces. As emphasized by many other studies on the foraging ecology of Red Knots, gizzard size is of great importance in diet and patch choice (Piersma *et al.* 1993, Piersma *et al.* 1999, Dekinga *et al.* 2001, van Gils *et al.* 2005a, 2005b, 2005c) and in the digestive process (Piersma *et al.* 2003, van Gils *et al.* 2003). As gizzard size can be rapidly and reversibly adjusted to prey quality (Piersma *et al.* 1999, Dekinga *et al.* 2001, van Gils *et al.* 2003),

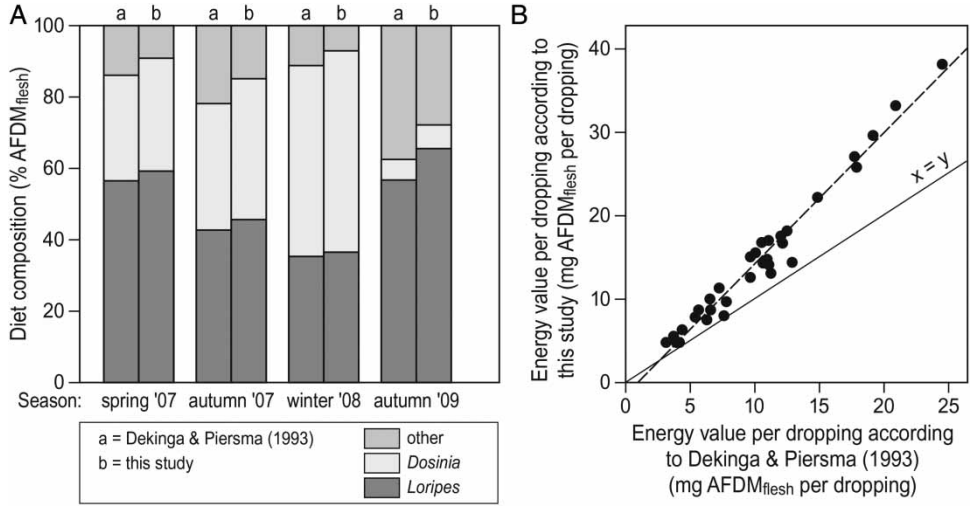

Figure 6. (A) Seasonal diet changes in terms of $AFDM_{flesh}$ in Banc d'Arguin ('other' in diet refers to other bivalves and gastropod species, where measurable fragments from droppings could be found). Per season the diet composition is calculated by multiplying DM_{drop} with 0.994 for bivalves or 1.267 for gastropods (from Dekinga & Piersma (1993), left bar per season) or 1.547 for *Dosinia isocardia* and *Loripes lucinalis* (derived from this study, right bar). (B) Energy value per dropping, expressed by using the Dekinga and Piersma correction factor (horizontal axis) and the factor obtained in our study (vertical axis).

gizzard sizes of our experimental birds might have changed during the experiments resulting in changed size-class preferences or reduced prey intake rates. However, it was not likely that gizzard size changed during the experimental period, because the birds were fed low-quality food (hard-shelled molluscs) every day and thus needed to maintain a large gizzard. However, between experimental trials, birds were fed with high-quality food, including unshelled *S. senilis* and commercial trout feed to meet the daily energy requirements (because we were unable to collect sufficient amount of molluscs for staple food). Thus, they might have reduced their gizzard with the prospect of high-quality food after the experiments. Nonetheless, intake rates did not change in the course of the experimental period (*Dosinia*: linear regression, $F = 0.5$, $P = .50$, $df = 21$) and for *Loripes*: linear regression, $F = 0.3$, $P = .59$, $df = 21$). We therefore conclude that there are no indications that the experimental birds changed their gizzard sizes during the feeding experiments in ways that would have affected their crushing performance.

Morphologically similar shells may have different crystallographic structures (Chateigner *et al.* 2000), affecting the degree that they would resist crushing. However, in the Wadden Sea, Dekinga & Piersma (1993) did not find a significant effect of prey species on either intercept or slope of the regressions of DM_{shell} on DM_{drop} within the group of bivalves. In Banc d'Arguin, we also found no significant difference between *Dosinia* and *Loripes*. This suggests a site-specific correction factor instead of a prey species-specific correction factor. Distinctive shell resistance to crushing can be caused by different predation regimes. Predators on bivalve molluscs, e.g. birds, fish and crabs (Carter 1968), can differ in abundance at different locations. More predation will result in thicker shelled molluscs (Edgell & Neufeld 2008). Shell thickness might also be attributed to latitudinal variation in water temperatures, either directly or indirectly. For example, Doyle *et al.* (2010) concluded that water temperature is indirectly responsible for shell thickness, because it facilitates the plastic phenotypic response to predation risk. Furthermore, ocean acidification due to increasing sea temperatures can impact the crushing capacities of bivalves by decreased calcification rates (Fabry *et al.* 2008, Rodolfo-Metalpa *et al.* 2011).

This is the first study showing that using the correction factors from Dekinga & Piersma (1993) may lead to somewhat biased diet reconstructions for Red Knots at Banc d'Arguin. In Bohai Bay, China, Yang *et al.* (2013) also carried out feeding experiments with captive Red Knots (subspecies *C. c. rogersi* and *C. c. piersmai*) to obtain a site-specific correction factor. A large fraction of the locally most important prey (the bivalve *Potamocorbula laevis*) was lost through the sieve, resulting in a DM_{shell}/DM_{drop} correction factor of 2.3. Consequently, previous studies that reconstructed Red Knot's diet based on factors derived from Dekinga & Piersma (1993), at other geographical locations than the Wadden Sea (e.g. Patagonia, Argentina, González *et al.* 1996; Roebuck Bay, Australia, Tulp & de Goeij 1994; and Miranda, New Zealand, Piersma 1991) may have to be re-examined.

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