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Cost–benefit analysis of mollusc-eating in a shorebird

II. Optimizing gizzard size in the face of seasonal demands

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Summary

Aiming to interpret functionally the large variation in gizzard masses of red knots *Calidris canutus*, we experimentally studied how the digestive processing rate is influenced by the size of the gizzard. During their non-breeding season, red knots feed on hard-shelled molluscs, which they ingest whole and crush in their gizzard. In three experiments with captive birds we tested predictions of the hypothesis that gizzard size, *via* the rate of shell crushing and processing, constrains intake rate in red knots (against the alternative idea that external handling times constrain intake rate). Gizzard size within individual birds was manipulated by varying the hardness of the diet on offer, and was confirmed by ultrasonography. The results upheld the ‘shell-crushing hypothesis’ and rejected the ‘handling time hypothesis’. Intake rates on with-shell prey increased with gizzard size, and decreased with shell mass per prey. Intake rates on

soft (without shell) prey were higher than on with-shell prey and were unaffected by gizzard size. Offering prey that were heavily shelled relative to their flesh mass led to energy intake rates that were marginally sufficient to balance the daily energy budget within the time that is naturally available in a tidal system. We predicted the optimal gizzard sizes that are required to either (1) balance energy income with energy expenditure, or (2) to maximise net daily energy intake. The gizzard mass of free-living red knots in the Wadden Sea is such that it maximises daily net energy intake in spring when fuelling for migration, while it balances energy budget throughout the remainder of the year.

Key words: gizzard, digestive constraint, intake rate, red knot, *Calidris canutus*, ultrasonography, optimization, phenotypic flexibility.

Introduction

Digestive systems respond rapidly and reversibly to specific local and temporal ecological circumstances (phenotypic flexibility; Piersma and Lindström, 1997; McWilliams and Karasov, 2001). For example, nutritional organs are enlarged when intake is high, such as when food is of poor quality (Starck, 1999), energy demand is high (Dykstra and Karasov, 1992; Speakman and McQueenie, 1996), or during fuelling for migration (Piersma and Gill, 1998; Piersma et al., 1999a,b). Organs are reduced when food quality is higher, when energy requirement is lowered, or when feeding becomes impossible (e.g. during long-distance flight). The size of such flexible organs appears to be a compromise between the costs and benefits that come with a certain size in a certain ecological setting. Increasing the size of nutritional organs leads to larger benefits in terms of energy intake (Hume and Biebach, 1996; Karasov and Pinshow, 2000; Lee et al., 2002; Karasov and McWilliams, in press), but also to larger costs in terms of maintenance (Lindström and Kvist, 1995; Piersma et al., 1996) and carrying them around (Houston, 1998). It is likely that the

ecological setting (e.g. food quality, energy expenditure or migratory phase) determines the specific organ size at which the benefits most outweigh the costs, but to the best of our knowledge such analyses have so far been lacking. In this study, we focus on how these energetic benefits vary with the size of one particular nutritional organ, the gizzard.

For two reasons we chose the red knot *Calidris canutus* as our model species. First, its nutritional organs and especially the gizzard are tremendously variable in size (Fig. 1; and see Piersma et al., 1999a,b). Second, the muscular gizzard plays a pivotal role in the bird’s feeding ecology, crushing the mollusc prey that are ingested whole (Piersma et al., 1993b). Changes in this organ’s size are likely to result in changes in shell-crushing and processing performance, thus changing energy intake rates. Given that mollusc prey contain little flesh relative to the amount of shell (5–20%), energy intake might readily be constrained by the rate at which shell material is processed by the gizzard, particularly when (1) gizzard size is small, and/or (2) the flesh-to-shell ratio (hereafter termed prey quality) is low.

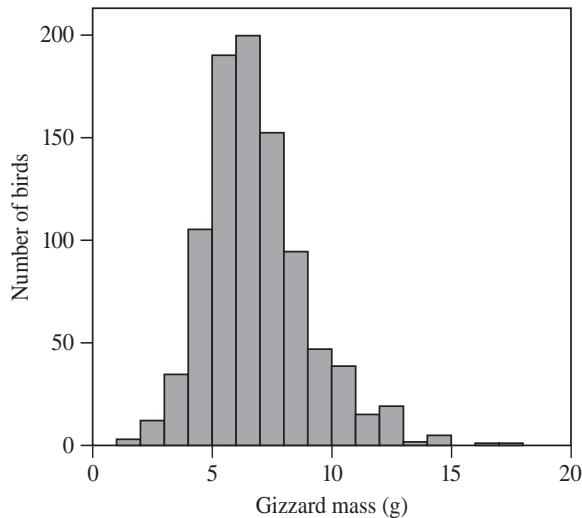


Fig. 1. Frequency distribution of gizzard mass (g) observed on free-living red knots throughout the years 1984–2002 in the Wadden Sea ($N=920$, of which 73 were obtained through dissection of carcasses and 847 through ultrasonography on live birds).

Phenotypic flexibility offers great experimental opportunities. As organ sizes vary within individuals, effects of such variability on performance can be studied within individuals (Piersma and Drent, 2003). If changes in organ size can be induced, one can test for effects by manipulating the size. Using ultrasonography, which is a non-invasive technique, Dekinga et al. (2001) showed that changes in gizzard size of red knots can be induced by the hardness of the food. Gizzards hypertrophied when knots were fed a hard-shelled diet (bivalves), and atrophied when on a soft diet (pellets). These changes were reversible and rapid; they occurred over a time scale of only 6–8 days.

Gizzard size was manipulated and confirmed by ultrasonography in individual red knots by changing the diet on offer. We then tested in three separate experiments the hypothesis that gizzard size constrains energy intake rate *via* the rate of shell crushing and processing. [Note that intake rate in this paper means intake over total time, which includes non-foraging activities such as digestive breaks. When feeding rates are high, red knots take short digestive breaks (20–300 s) at regular times (after 3–9 prey ingestions, depending on prey size). In the terminology of foraging theory, such intake rate over total time is called *long-term* intake rate, as opposed to *short-term* intake rate, which considers intake over foraging time only (see Fortin et al., 2002).] This ‘shell-crushing hypothesis’ predicts that (1) intake rates (prey s^{-1}) decline with the amount of shell mass per prey, (2) intake rates on with-shell prey types increase with gizzard size, (3) intake rates on intact prey (with shell) items are below those on shell-removed prey items, (4) intake rates on shell-removed items do not vary with gizzard size, and (5) intake rates on poor quality prey are insufficient to balance the energy budget within short daily available foraging times.

These predictions were tested against those of an alternative hypothesis inspired by foraging theory (Stephens and Krebs,

1986), the ‘handling time hypothesis’, which states that intake rates are constrained by the rate at which prey can (externally) be handled (i.e. the foraging activity between prey encounter and prey ingestion). It predicts that (1) intake rates (prey s^{-1}) are not different from the rate at which prey can be externally handled before being swallowed [note that handling rate (prey s^{-1}) is the inverse of handling time (s prey^{-1})], and (2) intake rates on with-shell prey do not vary with gizzard size.

Materials and methods

Gizzard size manipulations

Gizzard size was experimentally manipulated by changing the ‘hardness’ of the diet on offer, following procedures described by Dekinga et al. (2001). We aimed both to enlarge and reduce gizzard size. The success of the gizzard size manipulations was assessed by ultrasonography (Pie 200 ultrasound, Pie Medical Benelux BV, Maastricht, The Netherlands); all measurements were done by A.D.; for methods, see Dietz et al. (1999). Estimating gizzard mass (g) from carcasses with gizzard width (cm) measured by ultrasonography yielded a value for $r^2=0.70$ (Dietz et al., 1999).

Gizzard size manipulations in each of the three experiments were successful. Birds had larger gizzards when fed hard-shelled prey than when fed soft food (Fig. 2 and Table 1; $P<0.01$; see below for the time scale over which these changes took place). Corrected for the effect of diet hardness, gizzard mass differed among experiments ($P<0.05$), but not among individual birds ($P>0.15$).

Experiment 1

Using different prey species with different shell masses, we tested two predictions that follow from the ‘shell-crushing hypothesis’: (1) intake rates (prey s^{-1}) decline with a prey type’s shell mass, and (2) intake rates on with-shell prey types increase with gizzard size.

We created two groups, each of three birds *Calidris canutus* L., to which we randomly assigned individuals (because of logistic problems in collecting enough prey types for each bird we kept the total number of experimental birds at ‘only’ six). Before the start of the experiment, these groups were similar with respect to gizzard mass ($P>0.3$) and structural body size (principal component 1, PC1, from principal component analysis that included lengths of tarsus, toe, head and bill, $P>0.25$). All six birds were adult, captured with mist-nets in the Dutch Wadden Sea in 1994, 1995 and 1999. Ever since their capture, these birds had been housed in large in- and outdoor aviaries at the Royal Netherlands Institute for Sea Research (NIOZ, Texel, The Netherlands).

In order to manipulate gizzard size, one group was offered soft food (trout pellets; Trouvit, Produits Trouw, Vervins, France), the other group hard-shelled food (cockles *Cerastoderma edule*). This feeding regime was started 3 weeks before the experiment. Starting on 21 August 2000, we ran 36 trials with individual birds over 5 weeks (6 birds \times 6 prey types). Gizzard mass was confirmed by ultrasonographic

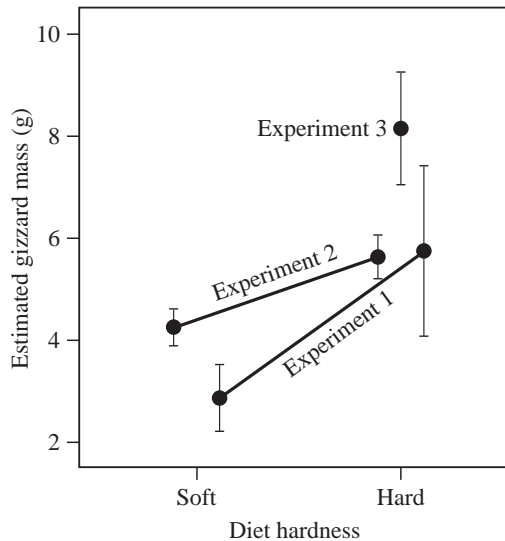


Fig. 2. Gizzard mass estimated ultrasonographically in the three experiments as a function of the hardness of the staple food on offer. Values are means \pm S.E.M. In all three experiments gizzard size manipulations were successful: the effect of the hardness of the diet on gizzard mass is significant ($P < 0.01$).

measurements at the beginning and at the end of the experiment, and remained at the particular level (small or large; $P = 0.55$), despite the fact that, during the short-lasting trials, the birds encountered prey that should have made them adjust their gizzard size (according to Dekinga et al., 2001).

The experiment took place on the isle of Griend in the western Dutch Wadden Sea ($53^{\circ}15'N$, $5^{\circ}15'E$). The close proximity to mudflats with a diverse array of prey species on offer facilitated the daily collection of prey items. Each group lived in a holding pen (2.5 m \times 1 m \times 0.5 m), which was placed under cover. Freshwater for drinking was always available.

Two bivalve prey species, which are commonly fed upon by red knots in the wild (Piersma et al., 1993a), were used in the experiment: the Baltic tellin *Macoma balthica* and the cockle. To incorporate size-related variation in shell mass, we offered different size classes of each prey species. The size criteria (all in mm) were: 5–7 (small), 9–11 (medium) and 13–15 (large), providing six different species-size categories or prey types. Prey length was measured to the nearest mm.

Prey items of only one type were offered in a single tray (0.2 m \times 0.15 m). Trials lasted 40 min, which yielded on average about 40 prey ingestions per trial. This number of ingestions should have been sufficient for intake rates to be constrained by rates of shell crushing, which commences after the gizzard is filled up, usually after 3–9 ingested prey (J. A. van Gils, unpublished data).

Shell mass (DM_{shell}) was measured by removing the soft, fleshy parts from a sub-sample of bivalves of each prey type class. Shells were put in crucibles and dried to constant mass for 3 days in a ventilated oven at 55 – $60^{\circ}C$, then weighed to the nearest 0.1 mg.

Intake rates were measured from video-recordings of the

Table 1. Nested analysis of variance testing for factors affecting gizzard mass in the three experiments

	d.f.	SS
Diet hardness	1	17.165
Experiment	2	20.935
Bird{group}	9	24.585
Error	10	14.355
r^2		0.859

All independent variables are categorical.

SS, sum of squares; d.f., degrees of freedom; bold figures indicate a significant contribution ($P < 0.05$).

trials. The full length of each trial was recorded using a Hi-8 video camera (SONY Nederland B.V., Badhoevedorp, The Netherlands) on a tripod, 1–2 m from the foraging bird. After each trial, the Hi-8 tape was copied to a VHS tape, to which a time-code was added. This enabled us to analyse the foraging behaviour from the VHS tapes by using 'The Observer' package (Noldus Information Technology, 1997). Tapes were analysed in slow motion (1/5 of recording speed) and behaviour was scored with an accuracy of 0.04 s and directly coded into digital files. We scored cumulative intake, handling times and non-foraging time (comprising standing still, preening and walking around).

Handling times were recorded to test the two predictions of the alternative 'handling time hypothesis' that intake rates were not governed by the rate of shell crushing but by the rate at which prey can externally be handled.

Experiment 2

Unlike experiment 1, where we offered different prey species and different sizes, we now offered only one bivalve prey species of just one size class. This was to eliminate variation in intake rate that was not due to variation in shell mass. We offered this one prey type either intact (as a hard-shelled prey) or with its shell removed (as a soft-bodied prey) in order to test three predictions that follow from the 'shell-crushing hypothesis': (1) intake rates on intact prey items are below those on shell-removed prey items; (2) intake rates on intact prey items increase with gizzard size; (3) intake rates on shell-removed items do not vary with gizzard size.

We used the same birds in the same groups as in experiment 1, except that one bird from the small-gizzard group of experiment 1 had to be replaced with one caught in the Dutch Wadden Sea in 1998. This did not change the pre-experimental similarity between groups in gizzard mass ($P > 0.9$) and structural body size ($P > 0.75$).

Gizzard size was manipulated by offering soft food (trout pellets) to one group, and hard-shelled food (blue mussels *Mytilus edulis*) to the other group. This feeding regime was initiated 4 weeks before the start of the experiment. Unlike in experiment 1, we now varied gizzard size *within* individuals by switching the diet between the two groups. This switch occurred in the middle of the experimental period, after which

we waited for 6 days to allow the gizzards to hypertrophy/atrophy to the new size (Dekinga et al., 2001). To check if gizzards had changed in size, every third day each individual's gizzard mass was estimated ultrasonographically. Starting on 3 May 2000, we ran 24 trials with individual birds in a period of 3 weeks (6 birds \times 2 treatments \times 2 gizzard sizes per bird).

The birds were housed in a climatized sea-container (5 m \times 2 m \times 3 m) at NIOZ. To ensure that the birds maximised their intake rates, we subjected them to cold-stress for the duration of the experiment at ambient temperatures of 3–4°C (cf. Klaassen et al., 1997), which should have increased their maintenance metabolism by at least 50% (Wiersma and Piersma, 1994) and thus their willingness to feed at maximum intake rates. The light-dark regime was kept constant (L:D=15 h:9 h). Each group of birds lived in a holding pen (2.5 m \times 1 m \times 0.5 m), which was kept clean continuously by seawater running over the floor. Freshwater for drinking was always available.

The prey type offered during the trials was the blue mussel (length=11.0 \pm 0.1 mm, mean \pm S.E.M., $N=149$). Mussels were collected by scraping them from basalt piers in the North Sea at Texel. After washing off most of the attached organic material, we sorted the mussels into different size classes by sieving through different mesh sizes. We kept the most abundant, medium-size class apart for the trials; the other size classes were offered as staple food. The mussels were stored in basins containing seawater of 5–12°C. We unshelled these prey by holding closed mussels in boiling water for 5–10 s, after which their valves opened, enabling us to remove the flesh with a pair of tweezers. Prey were offered in a single tray (0.6 m \times 0.4 m), that had running seawater through it to keep the mussels clean. To maintain methodological consistency with experiment 1, trials lasted 20 min each to guarantee about 40 prey ingestions per trial (note that these prey were generally eaten faster than the prey in experiment 1).

We used video-analysis as described for experiment 1 to measure intake rates and handling times. The latter measurement allowed us to test the two predictions of the alternative 'handling time hypothesis'.

Experiment 3

By offering a prey type with a very low flesh-to-shell ratio (0.09, equivalent to a metabolizable value of 1.44 kJ g⁻¹ DM_{shell}), we tested one of the predictions that follow from the 'shell-crushing hypothesis': knots feeding on poor quality prey experience difficulty in maintaining balanced energy budgets within the normally available foraging time (12 h per day in their intertidal habitat). We did not manipulate gizzard size in this experiment; instead we worked only with knots that had large gizzards. These were adult birds, caught in the Dutch Wadden Sea in 1997. They were given cockles as their permanent staple food 3 months before the experiment started. This ensured large gizzards in these birds, which was confirmed by ultrasonography 2 weeks before the experiment. Starting on 14 March 1998, we ran 28 trials with individual birds over 6 weeks (5 birds \times 3 treatments, with each

combination *in duplo* except for 2 trials). Prey quality at this time of year is poor (Zwarts, 1991).

The birds were housed in an indoor aviary (4.7 m \times 1.1 m \times 2.5 m) at NIOZ, in a constant environment with respect to light (15 h:9 h, L:D) and air temperature (16–20°C). Prey were divided equally across four trays (each 0.6 m \times 0.4 m) that had seawater running through them to keep the prey fresh and alive. Freshwater for drinking was always available.

In the experiment we used cockles, as they are the Wadden Sea's poorest quality prey (Zwarts, 1991) of size 11.4 \pm 0.1 mm (mean \pm S.E.M., $N=208$). These bivalves were collected on intertidal mudflats adjacent to the island of Texel. In the laboratory, the right size class (8–15 mm) was sorted out by sieving through different mesh sizes, followed by storage in basins containing seawater at 5–12°C.

The experimental treatment was the daily available time for foraging: either 2, or 6 or 16 h. We selected these times as they covered the extreme ranges of available daily foraging time in the tidally dictated circumstances in the wild. For all treatments, we always removed the food at the same time of day (20.00 h); thus we varied the length of the available foraging time by starting a feeding trial at different times of day (04.00 h, 14.00 h, 18.00 h). This enabled the birds to anticipate the time the food was on offer. In any other feeding schedule (random times or fixed starting times) the available daily foraging time could not have been anticipated by the birds.

Intake rate was measured as the total consumption during an entire trial divided by the length of a trial. As trials were long-lasting (2–16 h), we did not measure total consumption from video-analysis but from estimates of the initial number of prey offered minus the final number of prey remaining at the end of a trial. As we worked with many prey items per trial (up to 6000), initial and final prey numbers were estimated by weighing the fresh mass of a sub-sample of 100 cockles at the start and the end of each trial, respectively. These calibrations were then used to translate total fresh mass offered and remaining into total numbers. We used video-analysis only to measure handling times, by sampling random intervals of approximately 5 min h⁻¹, yielding about 10 prey ingestions per interval. This enabled us to test one prediction of the 'handling time hypothesis': intake rates (prey s⁻¹) are not different from handling rates (prey s⁻¹).

Shell mass was measured as in experiment 1. In addition, we measured ash-free dry mass of the prey's flesh ($M_{AFDflesh}$) by weighing dried flesh mass to the nearest 0.1 mg before and after incineration for 2 h at 550°C. This measurement was taken to calculate the intake rate required to cover the daily energy expenses for each treatment ($IR_{required}$ in prey s⁻¹). For a given treatment of n available foraging hours, $IR_{required}$ was calculated as:

$$IR_{required} = \frac{\left(\frac{24}{n}\right) R_{average}}{daM_{AFDflesh}}, \quad (1)$$

Table 2. Analyses of variance testing for factors affecting $\log(\text{intake rate})$ on shelled prey (experiment 1)

		Model							
		1		2		3		4*	
		d.f.	SS	d.f.	SS	d.f.	SS	d.f.	SS
DM_{shell}	Shell mass	1	17.508	1	17.508	0	17.102	0	17.102
G_j	Gizzard size	1	1.423	1	1.944	1	1.944	1	4.987
B_l	Bird	4	1.074	4	1.074	4	1.074	–	–
$DM_{\text{shell}} \times G_j$	Interaction	1	0.043	–	–	–	–	–	–
ϵ	Error	28	3.602	29	3.644	30	4.051	34	5.452
r^2			0.869		0.868		0.612		0.478

Log(shell mass) is a continuous variable in all models, and its effect is set to -1 in models 3 and 4.

Gizzard mass is a categorical variable in models 1–3 and continuous and log-transformed in model 4.

Bird is a categorical variable in all models.

SS, sum of squares; d.f., degrees of freedom; bold figures indicate a significant contribution ($P < 0.05$).

*Model 4 yields: $\log_{10}(IR) = -4.293 + 2.000 \log_{10} G - \log_{10}(DM_{\text{shell}})$, so $IR = 10^{-4.293} G^2 / DM_{\text{shell}}$, where IR is in prey s^{-1} , and both DM_{shell} and G are in g.

where d is the energetic density of 1 g M_{AFDflesh} (22 kJ; Zwarts and Wanink, 1993), and a is the assimilation efficiency (0.725; Piersma, 1994). R_{average} denotes the foraging time dependent average daily metabolic rate and is based on the cost of resting R_{rest} (1.665 W) and the heat increment of feeding R_{HIF} (1.082 W) estimated in the companion paper (Piersma et al., 2003):

$$R_{\text{average}} = R_{\text{rest}} + \left(\frac{n}{24} \right) R_{\text{HIF}} \quad (2)$$

Note that we did not take the costs of foraging into account (0.602 W; Piersma et al., 2003) since the prey were offered *ad libitum* in trays such that the birds did not have search for them. As a check upon this estimate for energy expenditure, we tested per treatment whether the birds lost weight on a daily basis.

General methodology in all three experiments

In each experiment we aimed to measure *maximum* intake rate (for a given gizzard size) in captive red knots in three ways. Firstly, we kept the birds at relatively low body mass (100–120 g) and starved them for at least 6 h before each trial to get them motivated and eager to eat. To keep the birds at constant low body mass we weighed them daily and adjusted the amount of food that they received accordingly. Secondly, we eliminated search time from the foraging process by offering unburied prey in dense, excess quantities. This ensures that intake rate will be constrained by either external handling times or by internal digestive processes (such as shell crushing in the gizzard). Thirdly, during each trial the test birds were feeding singly, so that intake rate would not be subject to interference competition. The birds not involved in a trial were kept in a separate cage for as long as a trial lasted.

Statistical analyses

In each experiment, a trial was used as the experimental unit, meaning that each trial yielded one data point on intake rate

that was used for statistical analyses. Intake rates were log-transformed to make them normally distributed. As some trials in experiment 1 yielded an intake rate of 0, we added 0.001 to all intake rates (prey s^{-1}) in this experiment to enable log-transforming 0 values (following Berry, 1987). Handling times were also log-transformed in order to normalise their distribution. All tests were performed using the General Linear Modelling procedure (GLM) in SYSTAT 10 (SPSS Inc., Chicago, IL, USA). The order in which trials were performed was randomised with respect to bird and treatment. Significance was accepted at $P < 0.05$.

Experiment 1

The following analysis-of-variance (ANOVA) model on intake rates IR on with-shell prey (model 1 in Table 2) tested two predictions of the ‘shell-crushing hypothesis’, that intake rate (prey s^{-1}) declines with a prey type’s shell mass (DM_{shell}), and that intake rate on with-shell prey types increases with gizzard size G_j (j =small, large).

$$\log(IR) = b_0 + b_1 \log(DM_{\text{shell}}) + G_j + B_l + b_2 \log(DM_{\text{shell}}) \times G_j + \epsilon, \quad (3)$$

where b_0 , b_1 and b_2 are regression coefficients, B_l is the effect of individual bird ($l=1-6$), and ϵ is an independent and identically normally distributed error. In this model we assume a linear relationship between $\log(IR)$ and $\log(DM_{\text{shell}})$ (with the slope varying with G_j via the interaction term). Next, we restricted one of the assumptions of this model, by leaving out the effect of the interaction (model 2 in Table 2):

$$\log(IR) = b_0 + b_1 \log(DM_{\text{shell}}) + G_j + B_l + \epsilon. \quad (4)$$

The model was further restricted by assuming that $b_1 = -1$ (i.e. that shell mass intake rate is constant across prey types; model 3 in Table 2):

$$\log(IR) = b_0 - \log(DM_{\text{shell}}) + G_j + B_l + \epsilon. \quad (5)$$

In the next model, we treated gizzard size as a continuous

variable (using the mean gizzard mass per bird per period; model 4 in Table 2):

$$\log(IR) = b_0 - \log(DM_{\text{shell}}) + b_3 \log G + \varepsilon. \quad (6)$$

These models were tested against each other using the extra sum-of-squares principle (Wetherill, 1986).

The first prediction of the 'handling time hypothesis', that intake rate (prey s^{-1}) for with-shell prey is not different from handling rate, can formally be written as $IR=1/H$, where H is handling time. When log-transformed, $\log(IR)=-\log(H)$, or $\log(IR)+\log(H)=0$. Thus, only for the trials on with-shell prey, we added $\log(H)$ to $\log(IR)$ for each trial, and tested the hypothesis that $b_0=0$ (where H is the least-square mean handling time for a given prey type). The second prediction of the 'handling time hypothesis', that intake rate for with-shell prey does not vary with gizzard size, is the opposite of the second prediction of the 'shell-crushing hypothesis' and was therefore tested by Equations 3–6.

Experiment 2

This ANOVA model tested three predictions of the 'shell-crushing hypothesis': (1) intake rate of intact prey items is below that of shell-removed prey items, (2) intake rate of intact prey items increases with gizzard size, and (3) intake rate of shell-removed items does not vary with gizzard size.

$$\log(IR) = b_0 + S_i + G_j + F_k + B_1\{F_k\} + G_j S_i + \varepsilon. \quad (7)$$

S_i is a dummy variable indicating whether the offered prey were with-shell or not (i =yes, no), F_k is the effect of flock ($k=1, 2$), and $B_1\{F_k\}$ is the effect of individual bird ($l=1, 2, 3$) nested within flock k . In addition, we tested whether gizzard mass and shell mass, using the parameters obtained in experiment 1 (Equation 6), correctly predicted $\log(IR)$ for with-shell prey. Thus, we tested the prediction that $\log(IR)=\log(IR_{\text{predicted}})$, where:

$$\log(IR_{\text{predicted}}) = b_{0,\text{exp1}} - \log(DM_{\text{shell}}) + b_{3,\text{exp1}} \log G. \quad (8)$$

The two predictions of the 'handling time hypothesis' were tested as described for experiment 1.

Experiment 3

The one prediction of the 'shell-crushing hypothesis' that we tested here, that knots feeding on poor quality prey can only marginally balance their daily energy budget within the normally available foraging time (12 h), was tested by the following ANOVA model (with and without interaction term):

$$\log(IR) = b_0 + B_1 + T_n + B_1 T_n + \varepsilon, \quad (9)$$

where T_n is the (categorical) effect of daily available time for foraging. For each daily available foraging time n we tested whether $b_0 + T_n = \log(IR_{\text{required}})$, where IR_{required} is given by Equation 1. In addition, we tested whether gizzard mass (8.13 ± 0.98 g, mean \pm S.E.M.; $N=5$) and shell mass, using the parameters obtained in experiment 1 (Equation 6), correctly predicted $\log(IR)$. Thus, we tested the prediction that $b_0 = \log(IR_{\text{predicted}})$, where $\log(IR_{\text{predicted}})$ is given by Equation 8.

The one prediction of the 'handling time hypothesis' that we could test, that intake rates are not different from handling rates, was tested as described for experiment 1.

Results

Experiment 1

Birds with large gizzards attained higher intake rates than birds with small gizzards ($P<0.001$), when taking the effect of shell mass into account ($P<0.001$; Fig. 3 and Table 2). This result suggests that it is shell mass that limits intake rate. Therefore, we tested whether intake rate (prey s^{-1}) declined with shell mass per prey using the observed relationships between shell mass DM_{shell} (mg) and shell length L (mm): for *Macoma* [$\log_{10}(DM_{\text{shell}}) = -2.076 + 3.873 \log_{10}(L)$, $N=236$, $r^2=0.876$, $P<0.001$]; for *Cerastoderma* [$\log_{10}(DM_{\text{shell}}) = -0.784 + 2.918 \log_{10}(L)$, $N=291$, $r^2=0.846$, $P<0.001$]. Indeed, among prey types, intake rate (prey s^{-1}) decreased with absolute shell mass per prey ($P<0.001$; models 1 and 2 in Table 2, Fig. 3) in such a way that on a log–log basis, the slope between these two variables did not differ from -1 ($P>0.05$; models 3 and 4 in Table 2, Fig. 3). This implies that each gizzard size-class can process a fixed amount of shell mass per unit time (0.24 and 2.58 mg s^{-1} , respectively, for small and large gizzard sizes). Replacing gizzard size-class by the actual gizzard mass (model 4) revealed that this shell mass intake rate actually increased quadratically with gizzard mass ($P<0.001$; Table 2). The explanatory power of these four statistical models does not differ (using the extra sum-of-square principle), hence the most parsimonious model, model 4, is preferred.

To test whether intake rates were constrained by handling time, we used the observed relationships between handling time H (s) and shell length L (mm) for *Macoma* [$\log_{10}(H) = -2.672 + 2.990 \log_{10}(L)$, $N=15$, $r^2=0.932$, $P<0.001$] and *Cerastoderma* [$\log_{10}(H) = -0.978 + 1.604 \log_{10}(L)$, $N=12$, $r^2=0.445$, $P<0.05$]. Handling rates ($1/H$) did not vary with gizzard size class and were significantly higher than intake rates ($P<0.001$ for both prey species; Fig. 3).

Experiment 2

The results of the previous experiment on multiple prey types implied that shell mass delimits intake rate. This interpretation is consistent with the results in the present experiment for a single prey type (Fig. 4; Table 3). (1) Intake rates on with-shell prey were higher for birds with large gizzards ($P<0.001$) and (2) rates were correctly predicted by the regression model from experiment 1 ($P>0.45$; Equation 8, using a DM_{shell} of 71.08 ± 3.84 mg, mean \pm S.E.M., $N=61$). (3) Intake rates on unshelled prey did not vary with gizzard size ($P>0.45$) and (4) for both gizzard size classes, intake rates on unshelled prey are higher than intake rates on with-shell prey ($P<0.001$), and (5) did not differ from the postulated maximum metabolizable energy intake rate ($P>0.05$; Kirkwood, 1983; Kvist and Lindström, in press). Finally, (6) intake rates were not as high as handling rates [$P<0.001$; handling times lasted

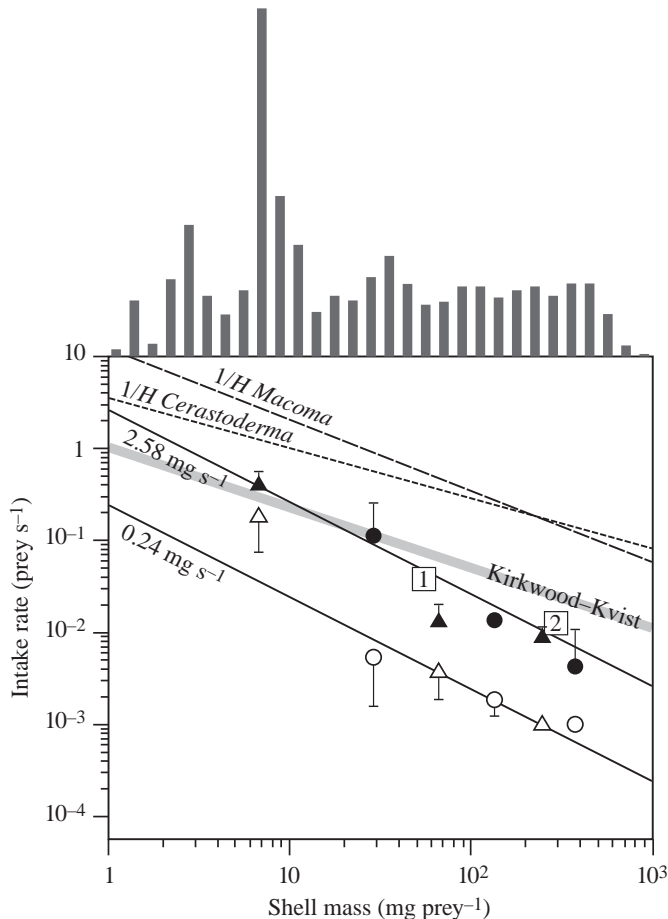


Fig. 3. Intake rates of the bivalve prey types in experiment 1 as a function of a prey type's shell mass (values are means \pm S.E.M.). Prey types are three different size classes of *Macoma* (triangles) and *Cerastoderma* (circles). Symbols are open for the small-gizzard birds and filled for the large-gizzard birds. Solid lines are the linear regression lines with fixed shell mass processing rates for each group of birds (0.24 and 2.58 mg s^{-1} , respectively, for the small- and large-gizzard birds; model 4 in Table 2). Broken lines give observed handling rates ($1/H$) for *Macoma* and *Cerastoderma*. Squares denote measurements on red knots in the field by (1) González et al. (1996) and (2) Zwarts and Blomert (1992). Grey diagonal 'Kirkwood-Kvist' bar indicates the constraint on metabolizable energy intake rate according to Kirkwood (1983) and Kvist and Lindström (in press; average of the two predictions is used). The bars on top of the graph denote the relative frequency distribution of shell masses of ingestible bivalve prey in the western Dutch Wadden Sea ($N=82\ 964$).

1.55 s on average, and were unaffected by bird (nested within flock, $P>0.9$), flock ($P>0.3$) and gizzard size ($P>0.2$).

Experiment 3

The observed intake rates on the poor prey type (Fig. 5) were not sufficient to cover the daily energy expenses when feeding for 2 h ($P<0.001$) or 6 h per day ($P<0.002$), but were sufficient when feeding for 16 h ($P>0.25$; Fig. 5). For these calculations (Equation 1) we used the mean M_{AFDflesh} (8.79 ± 0.32 mg, mean

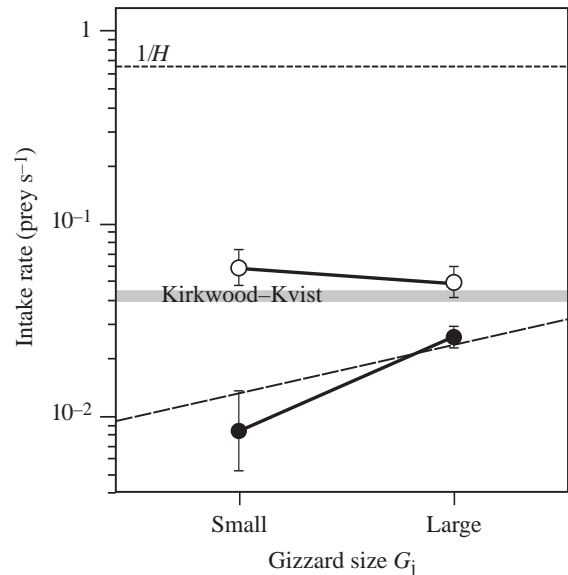


Fig. 4. Intake rate in experiment 2 as a function of gizzard size class (values are means \pm S.E.M.). Open circles indicate a soft diet of unshelled mussels was offered; closed circles, a hard diet of with-shell mussels was offered. The broken line gives observed handling rate ($1/H$). The broken diagonal line gives the intake rate for with-shell mussels predicted from shell mass, gizzard size and regression coefficients obtained in experiment 1. The grey bar gives the postulated maximum metabolizable energy intake rate [average of Kirkwood (1983) and Kvist and Lindström (in press)].

\pm S.E.M., $N=208$). This is consistent with the finding that the birds lost weight in the 2 h ($P<0.005$) and 6 h ($P<0.03$) treatment, but not when fed for 16 h ($P>0.1$). Intake rates did not vary with bird ($P>0.7$) and available foraging time ($P>0.95$; Table 4).

The two parameters from experiment 1 ($b_0=-4.293$ and $b_3=2.000$ in Equation 6) correctly predicted intake rate (prey s^{-1}) from gizzard mass and shell mass ($P>0.85$; broken line in Fig. 5). For this calculation (Equation 8) we used the mean DM_{shell} (97.54 ± 4.67 mg, $N=103$).

Intake rates (prey s^{-1}) were again much below handling rates ($P<0.001$; Fig. 5). Handling times lasted 2.93 s on average, and were unaffected by bird ($P>0.35$) and daily available foraging time ($P>0.3$).

Discussion

Intake rates for with-shell prey, which in every case were far below the handling rates (experiments 1, 2 and 3), were higher for birds with large gizzards than for birds with small gizzards (experiments 1 and 2). Intake rates on unshelled prey did not vary with gizzard size and were higher than intake rates on with-shell prey (experiment 2). Intake rates (prey s^{-1}) for with-shell prey declined with shell mass (experiment 1) in such a way that the gizzard-size-specific rate at which one unit of shell mass was processed was constant across prey types (models 3 and 4 in Table 2). Thus, the results of each of the

Table 3. Nested analysis of variance testing for factors affecting $\log(\text{intake rate})$ in experiment 2

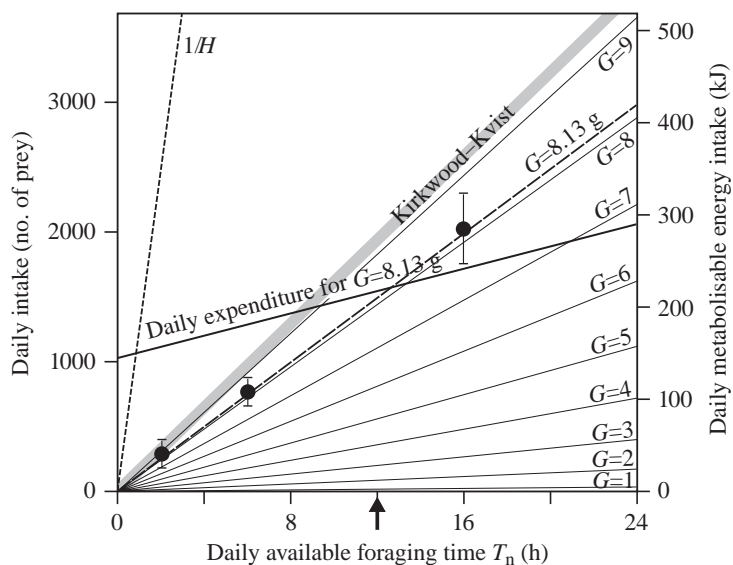
		d.f.	SS
S_i	With-shell	1	1.907
G_j	Gizzard size	1	0.256
F_k	Flock	1	0.277
$B_1\{F_k\}$	Bird{flock}	4	0.413
$G_j \times S_i$	Interaction	1	0.472
ϵ	Error	15	1.028
r^2			0.764

All independent variables are categorical.

SS, sum of squares; d.f., degrees of freedom; bold figures indicate a significant contribution ($P < 0.05$).

three experiments were consistent with the 'shell-crushing hypothesis' and refuted the 'handling time hypothesis'.

The fact that the gizzard can only process a fixed amount of shell mass per time unit suggests an underlying mechanism. From the breaking forces for various molluscs measured by Piersma et al. (1993b), it can be calculated that these forces scale linearly with shell mass (J. A. van Gils, unpublished data). It seems that a given gizzard size can only exert a given amount of work per unit time, i.e. the maximum power that a gizzard is able to generate seems to be responsible for the constraint on shell crushing rate. Alternatively, as the volumetric density of shell material is likely to be fairly constant across different prey types, the fixed amount of shell mass that can be processed per unit time could reflect the total volume of shell material that a full gizzard can contain. However, since the increase in gizzard mass is most likely to be due to increased muscle mass around the gizzard cavity, gizzard volume probably does not increase with gizzard mass (A. Purgue, personal communication; T. Piersma, personal observation), which makes the former 'force-idea' more likely.

Table 4. Analyses of variance testing for factors affecting $\log(\text{intake rate})$ in experiment 3

		Model			
		1		2	
		d.f.	SS	d.f.	SS
B_1	Bird	4	0.241	4	0.239
T_n	Foraging time	2	0.003	2	0.006
$B_1 \times T_n$	Interaction	8	0.112	–	–
ϵ	Error	13	1.322	21	2.434
r^2			0.507		0.092

All independent variables are categorical variables.

SS, sum of squares; d.f., degrees of freedom; none of the factors contributed significantly ($P > 0.05$).

Using these gizzard-size-specific rates of processing shell material (model 4 in Table 2), we can predict the ceiling on a knot's intake rate once we know its gizzard mass and the shell mass per prey (as done for experiments 2 and 3; broken lines in Figs 4 and 5, respectively). Furthermore, given the daily available feeding time (always ca. 12 h in the intertidal non-breeding habitat) and the energy content per prey, we can then predict whether a bird will be able to meet its daily energy requirements (experiment 3; Fig. 5). It is promising that two field estimates of intake rate (over total time: squares in Fig. 3; Zwartz and Blomert, 1992; González et al., 1996) are correctly predicted from the mean shell mass per prey in the diet and the gizzard mass estimated from relevant carcass analysis (T. Piersma, personal observation). This close match between field intake rates and model predictions shows the necessity of taking digestive constraints into account when using functional response models to predict long-term field intake rates. Functional responses based on encounter rates and handling times might correctly predict short-term intake rate (i.e. while foraging) from prey densities (Piersma et al., 1995), but long-

Fig. 5. Daily intake in experiment 3 increases as a function of the daily available foraging time T_n . Values are means \pm S.E.M.; intake is expressed both as number of prey (left axis) and in metabolisable energy intake (right axis). The rate of increase (i.e. the intake rate) is similar across the three treatments (2, 6 and 16 h; $P > 0.95$), and is correctly predicted by shell mass per prey and the flocks' average gizzard mass ($G = 8.13$ g; broken line based on the parameters of experiment 1; $P > 0.85$), and is much lower than the rate of prey-handling ($1/H$, broken line; $P < 0.001$). These observed intake rates were close to the postulated upper limit (grey bar; Kirkwood, 1983; Kvist and Lindström, in press). The thick solid line gives daily expenditure for $G = 8.13$ g. The experimental birds would just balance their daily energy budget when feeding for 12 h (arrow), which is exactly the time that is naturally available in their intertidal habitats. If the birds had had smaller gizzards (thin solid lines indicating gizzard mass G in g), they would have needed more time for this (even though their daily requirements would go down somewhat – this is not plotted here, but see Piersma et al., 2003).

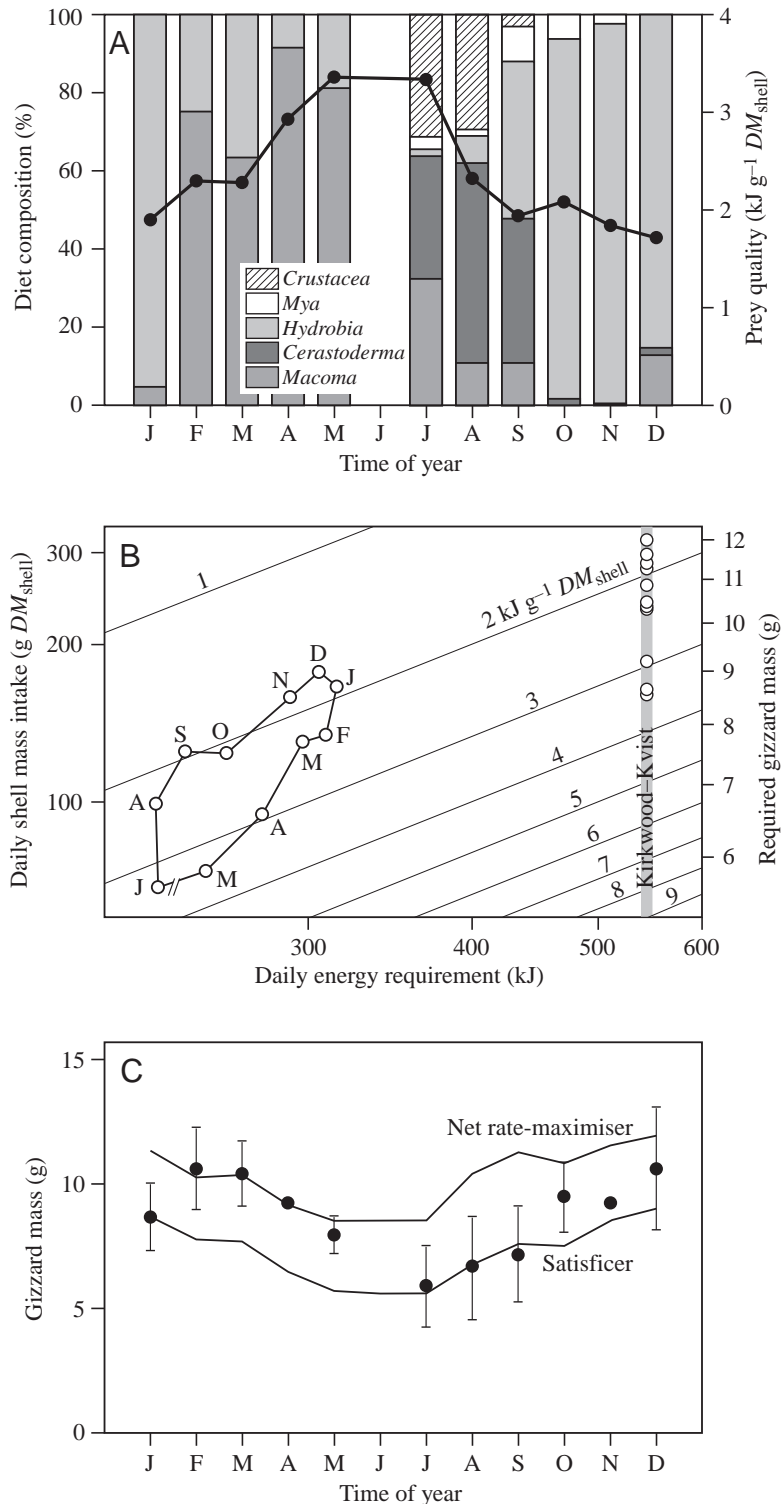


Fig. 6. (A) Diet composition (stacked bars scaled onto left axis; expressed as a percentage of total energy consumption) and the monthly-specific amount of flesh mass per prey type (Zwarts, 1991; not plotted here) determine the quality of the average ingested prey (filled circles scaled onto right axis, expressed as the amount of metabolizable energy per g shell mass, DM_{shell}). The data plotted here are for red knots living in the Wadden Sea (1988–2000). (B). For satisficing knots, prey quality (denoted by diagonal lines of equal prey quality) together with the daily amount of energy required to balance the energy budget in the Wadden Sea (horizontal axis) predict for each month the gizzard size (right axis) that is required to process the daily amount of shell material (left axis). Alternatively, for net rate-maximizing knots, prey quality together with the maximum amount of energy that can be assimilated on a daily basis (given by the vertical Kirkwood–Kvist bar) predict for each month the required gizzard mass. (C). Predicted gizzard masses for satisficing and net rate-maximizing red knots (lines) overlaid with data on gizzard masses of free-roaming red knots in the Wadden Sea in 1984–2002 (values are means \pm S.D.; $N=920$, of which 73 were obtained through dissection of carcasses and 847 through ultrasonography on live birds). Net rate-maximizing gizzards are found in spring (February–May), while satisficing gizzards are found throughout the remainder of the year (July–January).

(1) prey quality, (2) daily energy requirement and (3) daily available foraging time. The first parameter peaks at the start of the reproductive season of the prey (late spring in the Wadden Sea; Zwarts, 1991); the second parameter varies mainly with ambient temperature and wind speed (Wiersma and Piersma, 1994); and the third parameter is constant at 12 h day⁻¹ (Piersma et al., 1994). Since we know the monthly expectations in prey quality [Fig. 6A; based on Zwarts (1991) while taking into account diet composition; expressed as metabolizable kJ g⁻¹ DM_{shell}] and energy expenditure (Fig. 6B, calculated in Appendix), we can predict month-specific gizzard mass for red knots in living in the Wadden Sea (Fig. 6B,C). Depending on the criteria, the month-specific daily energy requirement can take two values. (1) If knots aim to *balance* their energy budget, daily energy requirement equals daily energy expenditure (i.e. satisficing; Nonacs and Dill, 1993). (2) If knots aim to *maximise* their daily net energy intake (i.e. net rate-maximization; Stephens and Krebs, 1986), their daily energy 'requirement' equals the physiologically

maximum daily gross energy intake, e.g. as derived by Kirkwood (1983) and Kvist and Lindström (in press). When not constrained by gizzard size, this maximum is presumably set by the size of other nutritional organs, such as the liver or the intestine (McWhorter and Martínez del Río, 2000); we found that intestine lengths in knots are constant throughout the year (T. Piersma, unpublished data). These two foraging

term intake rate (i.e. over total time) is likely to be governed by digestive capacity (van Gils et al., 2003). Having established that gizzard size and prey quality determine energy intake rates, we can now apply a reverse optimization routine to predict, for given environmental conditions, the gizzard size that is needed to fulfil the daily energy requirement. This prediction needs as input parameters

maximum daily gross energy intake, e.g. as derived by Kirkwood (1983) and Kvist and Lindström (in press). When not constrained by gizzard size, this maximum is presumably set by the size of other nutritional organs, such as the liver or the intestine (McWhorter and Martínez del Río, 2000); we found that intestine lengths in knots are constant throughout the year (T. Piersma, unpublished data). These two foraging

currencies lead to two unique predictions on optimal gizzard size for each month (Fig. 6B,C).

Data on gizzard mass of free-roaming red knots sampled in the Wadden Sea ($N=920$) fit a combination of these predictions remarkably well (Fig. 6C). Net rate-maximizing gizzards are found for red knots in spring, while satisficing gizzards are found throughout the remainder of the year (the best fit is found when modelling rate-maximization in February–May and satisficing in July–January; $r^2=0.23$, $P<0.001$). This shift in ‘foraging currency’ is consistent with seasonal changes in body mass and energy stores. Red knots accumulate large amount of energy stores in spring when preparing for their long-distance migrations, while in NW Europe their body mass remains quite stable during the rest of the year (Piersma, 1994). It is also consistent with an experimental study showing that red knots in spring maximised their net intake rate while exploiting food patches (van Gils et al., 2003). Furthermore, body mass increases a little in late autumn (October–December; Piersma, 1994), which is line with the gizzards being in between the satisficing and net-rate maximizing size at that time of year (Fig. 6C).

The fact that knots that are not building body mass appear to obey a satisficing strategy (but feed during the entire low tide period) fits the growing number of studies that show, in contrast to the original assumptions of optimal foraging theoreticians (Stephens and Krebs, 1986), that animals do not always forage at maximal intensities (Swennen et al., 1989; Norris and Johnstone, 1998; Iason et al., 1999). Note, however, that such satisficing behaviour should still be considered as part of an optimization process (see discussions in Stephens and Krebs, 1986; Nonacs and Dill, 1993), in which energy gain is traded against cost factors associated with foraging, such as the risk of parasite infestation or predation (Iason et al., 1999) or, in the case of probing waders, the risk of bill damage (Swennen et al., 1989; Norris and Johnstone, 1998). The way in which red knots balance their energy budgets adds another element to this discussion. Daily energy budgets could be balanced in periods shorter than 12 h per day if knots grew larger gizzards. For example, if knots in January had gizzards of about 14 g instead of the observed 9 g, their daily energy budget would be balanced when feeding for only 6 h per day. However, this would increase their average daily metabolic rate by 17% (due to higher maintenance and transport costs and reduced amounts of heat substitution). The fact that knots prefer to feed with smaller gizzards for the full extent of the low-tide period (12 h per day; Piersma et al., 1994), suggests that satisficing knots aim to minimise their overall rate of energy expenditure, perhaps in order to maximise lifespan by minimizing the level of free radicals (Daan et al., 1996; Deerenberg et al., 1997; Tolkamp et al., 2002).

To conclude, gizzard size sets the maximum processing rate of shell material, and the constraint on a knot’s daily energy intake is therefore a function of (1) the amount of flesh per g shell material (i.e. prey quality), (2) gizzard size and (3) the daily time available for foraging. Seasonal variation in prey quality and required energy consumption (being a function of

ambient temperature and migratory phase) together explain the seasonal variation in gizzard mass of red knots living in the Wadden Sea.

Appendix

Optimal gizzard size for satisficing red knots

Satisficing red knots aim to balance energy expenditure with income on a daily basis. We therefore need to equate both income and expenditure as a function of gizzard size to predict the optimal gizzard size for satisficing red knots.

Income

From the results of experiment 1 (model 4, Table 2) we know that energy intake rate IR (W) depends on prey quality Q (J metabolizable energy per g shell mass) and gizzard mass G (g) in the following form:

$$IR = Q \times 10^{-4.293} \times G^2. \quad (A1)$$

Expenditure

From Piersma et al. (1996) we know that basal metabolic rate (BMR, in W) scales linearly to lean mass (L) in the following form:

$$BMR = 0.0081L - 0.046. \quad (A2)$$

We know that in red knots gizzard mass G and intestine mass I are highly correlated in a 1:1 relationship (see table 3 in Piersma et al., 2003), thus:

$$G = I. \quad (A3)$$

If we define L_{without} as lean mass without gizzard and intestine mass (set to 100 g in this study), then we can replace L in Equation A2 by $L_{\text{without}}+2G$ to equate BMR as a function of gizzard mass.

From Kvist et al. (2001), we know that the metabolic rate while flying R_{fly} (W) scales to total body mass B (g) in the following way (assuming that the birds in that study had BMR values of 0.95 W):

$$R_{\text{fly}} = 10^{0.39} \times B^{0.35} - 0.95. \quad (A4)$$

Since total body mass equals lean mass plus fat mass F (set to 0 g in this study) we can replace B by $L_{\text{without}}+2G+F$ to equate R_{fly} as a function of gizzard mass.

From the accompanying paper (Piersma et al., 2003) we know that metabolic costs of foraging amount to 0.602 W. If we assume that these costs are the sum of the cost of probing (R_{probe}) and the cost of walking (R_{walk}), we can predict how foraging costs will vary with gizzard mass. Bruinzeel et al. (1999) show that the R_{walk} (W) equals:

$$R_{\text{walk}} = v840 \left(\frac{B}{1000} \right)^{2.90}, \quad (A5)$$

where v is velocity (m s^{-1}). We need to replace B by $L_{\text{without}}+2G+F$ to equate R_{walk} as a function of gizzard mass.

Since the mean mass of the birds during the DLW experiment (Piersma et al., 2003) was 122.2 g and they walked at a mean velocity of 0.072 m s⁻¹, R_{probe} is estimated at 0.47 W.

Piersma et al. (2003) show that the metabolic costs of digesting (i.e. the heat increment of feeding, HIF) amount to 1.082 W. Assuming that HIF increases linearly with the amount of flesh that is digested, and given the observed flesh intake rate of 0.208 mg s⁻¹ (fig. 1B in Piersma et al., 2003), the HIF cost of digesting 1 g of M_{AFDflesh} equals 5195 J. Since we know how intake rate (prey s⁻¹) scales with gizzard mass (Equation A1), HIF (W) depends on gizzard mass G (g) in the following form:

$$\text{HIF} = 5195 M_{\text{AFDflesh}} \left(\frac{10^{-4.293} \times G^2}{DM_{\text{shell}}} \right). \quad (\text{A6})$$

The month-specific thermostatic costs in the Wadden Sea range from 1.64 W in August to 2.93 W in January (Wiersma and Piersma, 1994). Some of the heat generated in other cost-components can substitute for this thermoregulatory heat, which makes life considerably cheaper. All of the heat generated by BMR (Scholander et al., 1950; Wiersma and Piersma, 1994) and presumably also all of the HIF heat can be used for thermoregulatory purposes (like BMR, HIF is after all generated in the core of the body). From Bruinzeel and Piersma (1998) we calculated that about 30% of the heat generated due to walking substitutes for thermostatic heat.

Daily energy income and expenditure depends on the time devoted to all of the above-mentioned activities. Of course, BMR and thermoregulatory costs are expended for 24 h per day. Red knots in the Wadden Sea devote about 1 h per day to flight (between roosts and feeding sites) and about 12 h per day to foraging (i.e. walking, probing, and HIF; Piersma et al., 1993a). Taking these time budgets into account while equating income with expenditure solves for optimal gizzard mass in satisficing knots.

Optimal gizzard size for net rate-maximizing red knots

Net-rate-maximizing knots should aim for gizzard sizes that process food at the physiologically maximum rate (see fig. 4 in Piersma et al., 2003). According to Kirkwood (1983) and Kvist and Lindström (in press), this should yield red knots a gross income of 544 kJ on a daily basis. Using the gizzard-size-dependent function for intake rate (Equation A1), and again assuming that knots feed for 12 h per day on prey of quality Q (J g⁻¹ DM_{shell}), the optimal gizzard mass for net-rate-maximizing knots $G_{\text{net-rate}}$ (g) therefore equals:

$$G_{\text{net-rate}} = \sqrt{\frac{544 \times 1000}{12 \times 3600 \times Q \times 10^{-4.293}}}. \quad (\text{A7})$$

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