RESEARCH ARTICLE

Economic design in a long-distance migrating molluscivore: how fast-fuelling red knots in Bohai Bay, China, get away with small gizzards

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SUMMARY

We carried out an observational and experimental study to decipher how resource characteristics, in interaction with the predator's phenotype, constrain a fitness-determining performance measure, i.e. refuelling in a migrant bird. Two subspecies of red knot (*Calidris canutus rogersi* and *C. c. piersmai*) use northern Bohai Bay, Yellow Sea, China, for the final prebreeding stopover, during their 10,000–15,000 km long migrations between wintering and breeding areas. Here, they feed on small bivalves, especially 2–7 mm long *Potamocorbula laevis*. With an average stay of 29 days, and the need to store 80 g of fat for the onward flights to high-Arctic breeding grounds, red knots need to refuel fast. Using existing knowledge, we expected them to achieve this on the basis of (1) prey with high flesh to shell mass ratios, (2) large gizzards to crush the ingested molluscs, or (3) a combination of the two. Rejecting all three predictions, we found that red knots staging in Bohai Bay had the smallest gizzards on record (4.9±0.8 g, mean ± s.e.m., *N*=27), and also found that prey quality of *P. laevis* is much lower than predicted for the measured gizzard size (i.e. 1.3 rather than the predicted 4.5 kJ g^{-1} dry shell mass, DM$_{shell}$). The estimated handling time of *P. laevis* (0.2 s) is much shorter than the observed time between two prey ingestions (0.7 s), indicating that prey handling time is no constraint. Based on field observations of dropping rates and on indoor digestion trails, the shell processing rate was estimated at 3.9 mg DM$_{shell}$ s^{-1}, i.e. three times higher the rate previously predicted for red knots eating as fast as they can with the measured gizzard size. This is explained by the small and easily crushed *P. laevis* enabling high processing rates. As *P. laevis* also occurred in high densities, the metabolizable energy intake rate of red knots with small gizzards at 5 J s^{-1} was as high as at northward staging sites elsewhere in the world. Currently, therefore, food characteristics in Bohai Bay are such that red knots can refuel fast whilst economizing on the size of their gizzard. These time-stressed migrants thus provide an elegant example of symmorphosis.

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INTRODUCTION

Many aspects of an organism's phenotype appear fine-tuned with respect to environmental conditions (Piersma and van Gils, 2011), and so the performance of organisms in various ecological contexts depends on both environmental conditions and their particular ability to respond. Long-distance migration provides cases where a single performance measure is strongly selected for, i.e. (re-)fuelling rate (Zwarts et al., 1990; Baker et al., 2004; Piersma et al., 2005; Atkinson et al., 2007; Morrison et al., 2007; Verkuil et al., 2012). The deposition of fat and protein stores (and even calcium stores when females prepare for egg laying) (Piersma et al., 1996) is a function of the availability of resources, but as food must be digested, the size of the digestive machinery may provide another layer of constraint (van Gils et al., 2003; McWilliams et al., 2004; van Gils et al., 2006a). According to symmorphic design rules, organs should grow to sizes to satisfy but not exceed the requirements (Taylor and

Weibel, 1981). Long-distance migrating birds with high energy demands at stopover sites need to avoid excess digestive capacity. Comparative data may show the extent to which they do.

Red knots (*Calidris canutus*) are long-distance migrants that, away from the tundra breeding grounds, mostly depend on molluscs found on intertidal mudflats (Piersma et al., 2005; Piersma, 2007). Hard-shelled prey items are swallowed whole and crushed in muscular gizzards (Piersma et al., 1993a; Piersma et al., 1995; Piersma et al., 2004; van Gils et al., 2005a). The crushed shell material is evacuated through the relatively heavy gut (Battley and Piersma, 2005). This processing of such hard-shelled prey may lead to constraints on energy intake being internal (digestive bottleneck), rather than external (handling times) (van Gils et al., 2003).

Red knots adjust the mass of their digestive organs in the course of the annual cycle relative to changing environmental contexts and internal states (Piersma and Lindström, 1997; Dietz et al., 1999;

Piersma et al., 1999a; Piersma et al., 1999b; McWilliams and Karasov, 2001; van Gils et al., 2006a). In situations where red knots eat hard-shelled prey, the digestive rate model (DRM) has been successful in explaining diet choice, patch choice, and energy intake rates as a function of gizzard size and prey quality (flesh to shell mass ratios) (van Gils et al., 2003; van Gils et al., 2005a; van Gils et al., 2005b; van Gils et al., 2005c). Red knots generally enlarge their gizzard when food quality is low and reduce it when food quality is high (Dekinga et al., 2001; van Gils et al., 2003). However, for *Calidris canutus canutus* and *C. c. islandica* in the East Atlantic Flyway, and for *C. c. rufa* in the West Atlantic Flyway, a doubling of food demands during fuelling at staging sites compared with non-fuelling situations did not lead to measured increases in gizzard size because the birds selected areas that seasonally offered high quality prey (van Gils et al., 2005a). In so doing, the red knots avoided investment in the hypertrophy of gizzards upon arrival at staging areas.

Two other subspecies of red knot, *C. c. piersmai* Tomkovich 2001 and *C. c. rogersi* (Mathews 1913), migrate along the East Asia–Australasian Flyway (Piersma, 2007; Rogers et al., 2010). During the northward migration towards the high Arctic, from 'wintering' areas in northwest Australia and New Zealand, respectively, the great majority of both subspecies make a refuelling stop in northern Bohai Bay, Yellow Sea, China (Battley et al., 2005; Rogers et al., 2010; Yang et al., 2011; Hua et al., 2013). Although *C. c. piersmai* on average migrated 1–2 weeks later than *C. c. rogersi*, both subspecies staged for an average of about 29 days (Rogers et al., 2010). Based on the existing version of the DRM (van Gils et al., 2003; van Gils et al., 2005a; van Gils et al., 2005b; van Gils et al., 2005c), and on observations on departure timing and gizzard size in northwest Australia by the *C. c. piersmai* subspecies, Battley and colleagues (Battley et al., 2005) made a set of interdependent predictions on rates of fuelling, gizzard size and prey quality during the Yellow Sea stopover. They predicted that with the little time available to make organ adjustments upon arrival in the Yellow Sea, the relatively small gizzards carried from northwest Australia could be maintained only if they could find high-quality prey in China.

In this paper we tested the predictions of Battley and colleagues (Battley et al., 2005) by examining gizzard size, prey quality and refuelling rates on the basis of field observations and laboratory experiments. We found that the theoretical scaffolding provided by Battley and colleagues (Battley et al., 2005) requires qualification, and also discovered that 'crushing power' (the force to break ingested shellfish) is a hitherto unappreciated prey quality variable that needs incorporation in future molluscivore foraging models. Our investigation was structured according to the methodology used, as follows. (1) We compare prey quality (based on diet analyses) and gizzard size (based on dissections) with the quantitative predictions of Battley and colleagues (Battley et al., 2005). (2) We evaluate shell processing and energy intake rates of red knots in Bohai Bay. (3) We try to explain these rates in terms of a new variable in the argument, the small breaking force of ingested shellfish.

MATERIALS AND METHODS
1. Testing the predictions
Based on a digestive rate model that includes gizzard size and prey quality (ratios of flesh to shell mass), Battley and colleagues (Battley et al., 2005) predicted that if red knots (*C. c. piersmai*) were to fuel successfully in the Yellow Sea, they would need (1) to enlarge their gizzards if prey quality was lower than 2–3 kJ g^{-1} dry shell mass (DM$_{shell}$), (2) to maintain gizzard sizes found in Australia (5.9 g)

(Battley et al., 2000) if the prey quality values they encountered in the Yellow Sea were higher than those in NW Australia (*ca.* 3.5–4.5 kJ g^{-1} DM$_{shell}$ rather than 2.2 kJ g^{-1} DM$_{shell}$), or (3) to reduce gizzard size the prey quality in the Yellow Sea exceeded 4.5 kJ g^{-1} DM$_{shell}$. We examined these predictions on the basis of measurements of diet composition, the quality of the prey consumed and the gizzard sizes of red knots during their stay in Bohai Bay in April–May 2008.

Diet composition
During the northward migration period in 2008 we studied red knots and their mollusc food at three sites in northern Bohai Bay (Luannan County, Hebei Province): (1) on the mudflats at Zuidong (39°1′N, 118°18′E, 30 April, 13 and 29 May 2008), (2) at Nanpu (39°4′N, 118°12′E, 29 April, 14 and 26 May 2008), and (3) at Beipu (39°9′N, 118°8′E, 7, 21 and 27 May 2008). The study area is described in detail elsewhere (Rogers et al., 2010; Yang et al., 2011).

During low tide we followed flocks of red knots and took dropping samples after birds had fed at a location for at least 40 min (food retention time in the digestive tracts of red knots is 20–50 min) (Piersma, 1994). We collected a total of nine samples of 60 droppings each, which were stored at −20°C. In the laboratory, the samples were processed as outlined previously (Dekinga and Piersma, 1993). Briefly, after being washed over a 0.3 mm mesh and dried at 60°C for 3 days, shell fragments were sorted into different species and the size distribution of each species was reconstructed from unbroken hinges (to the nearest 0.1 mm; see Table 1). The shell fragments were so numerous that subsamples were taken for species identification (10% of total broken shell mass) and hinge height measurements (*ca.* 10% of total hinge number). Note that the loss of small hinges through the 0.3 mm mesh was corrected for, at least in the main prey *Potamocorbula laevis*, by correction factors derived from the digestion experiments reported in section 2 ('Energy intake rates') of Materials and methods. Diet was determined by mass composition of different shell species on the basis of shell mass multiplied by the size-specific ash-free dry mass of flesh/shell dry mass ratios (Dekinga and Piersma, 1993).

We measured the density of accessible prey at locations where the diets were ascertained from dropping samples by taking eight cores of 1/56 m^2. Cores were taken to a depth of 4 cm (i.e. within the reach of the bill of red knots) (Zwarts et al., 1992; Zwarts and Blomert, 1992) and sieved through a 1 mm mesh. Snails were sampled by a smaller core (1/267 m^2) to a depth of 4 cm and using a finer mesh (0.5 mm). All potential prey items retained on the sieve were stored at −20°C for later analysis.

Table 1. Diet reconstruction

Bivalve species	Relationship	R^2	N
Potamocorbula laevis (left shell)*	$L=10.392\times T+0.869$	0.95	66
Moerella iridescens (both shells)	$L=19.16\times T-1.548$	0.95	202
Mactra quadrangularis (both shells)	$L=10.26\times H+17.17$	0.98	161
Theora fragilis (both shells)	$L=25.94\times T-8.41$	0.72	61
Cyclina sinensis (both shells)	$L=7.302\times T-5.888$	0.95	28
Musculus senhousei	$L=69.86\times H-50.11$	0.80	30
Stenothyra glabra	$S=1.95\times W-6.017$	0.87	38
Nassarius variciferus	$S=2.35\times W+1.684$	0.91	26

The relationship between height of hinge and top (*T*, mm) or hinge only (*H*, mm) and shell length (*L*, mm) for each bivalve species, and the relationship between shell height (*S*, mm) and width of the last whorl (*W*, mm) for univalve species in the diet of red knots are shown.
*See supplementary material Fig. S1.

In the laboratory, items were counted and species and size (to the nearest 1 mm) were determined, enabling determination of the ingestible fraction (Piersma et al., 1993a; van Gils et al., 2005b; Kraan et al., 2009). Subsamples were taken for measuring dry shell mass (DM_{shell} for bivalves and DM_{total} for gastropods, g) and ash-free dry mass ($AFDM_{flesh}$ for bivalves and $AFDM_{total}$ for gastropods, g). The shell and flesh of bivalve samples in the subsample were divided and weighed after being dried at 60°C for 3 days. The flesh was incinerated at 550°C for 5 h and ash-free dry mass measured (Piersma et al., 1993b; van Gils et al., 2006b; Kraan et al., 2010). In snails, flesh was not separated from the shell. Only the ingestible fraction of the prey (shell lengths <20 mm) was used in estimates of available prey (Zwarts and Blomert, 1992).

Prey quality

The size-specific prey quality of the main prey (*P. laevis*) (Q, kJ g^{-1} DM_{shell}) was calculated as: $Q=d×a×AFDM_{flesh}/DM_{shell}$, where d is the energetic density of the flesh (22 kJ g^{-1} AFDM) (Zwarts and Wanink, 1993) and a is assimilation efficiency (0.8) (Kersten and Piersma, 1987; Piersma, 1994). As the relationship between prey quality and length is not linear, we transformed the data logarithmically. Based on 242 samples, $Q=10^{0.226}×L^{-0.22}$ (where L is shell length; $R^2=0.86$, $F_{1,240}=1600$, $P<0.0001$).

Gizzard size

After capture with wind-assisted clapnets (Choi et al., 2009) during low tide on mudflats at Zuidong in April–May 2008, red knots were individually ringed and their body dimensions and mass measured. Twenty-seven birds were killed by accident, or were injured and rapidly killed by thoracic pressure (see Hua et al., 2013). Birds were weighed to the nearest 0.1 g as soon as possible after capture. Carcasses were sealed in airtight plastic bags and stored at −20°C before being transported to the laboratory for dissection. The dissection procedures followed those described elsewhere (Piersma et al., 1999b; Battley et al., 2000). Fresh masses of gizzards were taken to the nearest 0.1 g on an analytical balance. The corpses were not assigned to subspecies.

Data analysis

To establish selection by red knots of bivalve prey species, and of the size of *P. laevis* (the most common prey of knots), Ivlev's (s)electivity index E (Jacobs, 1974) was calculated as: $E_i=(r_i−p_i)/(r_i+p_i)$, where r_i is the percentage of bivalve prey species i in the diet (biomass of species i/total biomass of bivalves in the diet×100) and p_i is the percentage of this species in the top layer of mud. The index E for size selection of *P. laevis* was calculated from the number of *P. laevis* present in the mud and the faeces following the same equation, where r_i is the percentage of prey of size class i in the diet (number of size class i/total number of prey individuals in the diet×100) and p_i is the size-specific percentage of the prey in the mudflats. E values may range from −1 (no use) to 1 (high selection), or, when E equals 0, no selection.

We tested gizzard size for significant variance among dates, body masses and sex using general linear models (GLM). We also tested the difference in biomass proportions of bivalve species available in the mudflats and eaten by red knots using Chi-square tests. All analysis was performed using SYSTAT13 (SYSTAT Software Inc., Chicago, IL, USA).

2. Energy intake rates

In Bohai Bay, the prey of red knots are so small that intake rates cannot be measured directly from rates of ingestion (e.g. Zwarts et al., 1996) (see section 1, 'Testing the predictions', Materials and methods). Instead, to assess energy intake rates in the field, we measured dropping intervals (González et al., 1996). We also conducted a digestion experiment to determine the correction factor for converting sieved shell mass (dropping fragments) to ingested shell mass. It was then possible to estimate energy intake and shell processing rates on the basis of the time interval between droppings and the average shell mass in each dropping.

Dropping intervals

We measured intervals between two consecutive defecations at the study sites in spring 2009 (see González et al., 1996; Piersma et al., 2004). Measurements were independent, in that we measured the time between successive defecations for a single bird before moving on to the next. Intervals were recorded only when the tails of individuals under observation remained towards the observer during the interval. Small interruptions (due to a sudden brief turn, or the sight line being blocked for a fraction of a second as another bird passed in view) were accepted. Less than 20% of total measured dropping intervals were interrupted for too long and were dismissed (note that this may cause a small bias as long intervals have a higher chance of being interrupted). Birds observed were identified to subspecies (*C. c. piersmai* or *rogersi*) on the basis of their plumage characteristics (Hassell et al., 2011).

Digestion experiment

Twelve red knots were caught on the mudflats at Zuidong (39°01′N, 118°19′E) on 25–26 May 2010. Their body masses varied between 106 and 160 g at capture. Only the five birds with body masses below 129 g at capture would eat in captivity. These five birds included four adult *C. c. piersmai* and a second calendar-year bird of unknown subspecies. The individuals that refused to eat were released in the field after a few days, as were the other individuals at the end of the experiment.

During the experiments each bird lived in an individual cage (0.6×0.4×0.4 m) placed on the floor of an apartment room where the natural daylight cycle and outdoor temperatures were maintained. Cages were put side by side in two lines, and the whole assembly of cages was covered by a light cloth so that the birds could see each other, but were visually separated from the disturbance of people entering and leaving the room. To help them adjust to the indoor conditions, birds were exposed to two calmly moving and talking people for an afternoon and were given water and fresh natural food for 1 day before the trials. In this setting the knots quickly became adjusted to the close presence of people.

The experiment was conducted from 26 May to 1 June 2010. All 15 trials started at 14:00 h–20:00 h and lasted for 20–24 h. The bottom of each cage was covered with a plastic net with 1 cm mesh. Droppings fell down through the mesh to a plastic plate below. Fresh *P. laevis* of all sizes were offered as food. Between 100 and 260 g *P. laevis* was given per individual trial, adjusting the amounts to the mass of prey retained from the previous trial. *Potamocorbula laevis* and water were offered separately in small cups. Before each trial, samples of the prey on offer were taken (2–5 g sample^{-1}). The fresh mass of these samples and the total amount of food for each bird were weighed. After a trial, all prey remaining in the cups and spilled on the plates were collected, as were the droppings. All samples were frozen at −20°C for later analysis. Note that upon ending the trials on the second day, some birds were still processing food, so that during the analysis we had to account for non-empty guts (estimated as the intercept of the regression of mass of ejecta on mass of ingesta, see below).

In the laboratory, droppings were dried at 60°C for 3 days, weighed and then sieved over 0.3 mm mesh, and the dry mass weighed. For the droppings collected after each trial, subsamples of 200 measurable hinges were measured (to the nearest 0.1 mm) among the dropping fragments on the 0.3 mm mesh. For the prey samples, *P. laevis* were counted and the size distribution measured (to the nearest mm). Flesh and shell were separated in a subsample of *P. laevis* specimens, and the measurement of dry shell mass (DM_{shell}, g) and ash-free dry mass ($AFDM_{flesh}$, g) was carried out described in section 1 ('Testing the predictions', Materials and methods). There were left-over or spilled-out *P. laevis* in some trials. The remaining prey were counted, and accounted for less than 5% of the total number of the food given in every trial; we took them into account for the correction of ingested shell mass, but when correcting for a bias in shell size reconstructions we obviously did not incorporate them as ingested prey items.

In our analysis we only included trials during which birds ate more than 5 g fresh mass of *P. laevis*. With an overall average starting body mass of 118 g (s.d.=15 g), the birds ate an average of 178 g fresh mass of shellfish, and on average showed a body mass change of 0.3 g during the experiments (range 4–5 g). With an average mass gain of 4 g or more in the wild (Z.-J.M. and N.H., unpublished data), the birds were not processing at as a high rate as free-living birds in Bohai Bay would have done. However, we have no reason to think that this would affect the degree of crushing of the shellfish, the variable of interest here.

Data analysis

To establish the correction factor needed to 'translate' weighed amounts of sieved and sorted *P. laevis* shell fragments into ingested shell masses, we determined the ratio previously denoted r (Dekinga and Piersma, 1983) of dry mass of ingested shell ($DM_{shell,ingested}$) to dry mass of droppings retained on a 0.3 mm mesh ($DM_{droppings}$):

$$r = \frac{DM_{shell,ingested}}{DM_{droppings}} . \qquad (1)$$

$DM_{shell,ingested}$ was calculated from the proportion of dry shell mass of each prey sample ($DM_{shell,sample}$) to the flesh mass of the same sample ($FM_{food,sample}$) and flesh mass of total food ingested in the trial (FM_{food}):

$$DM_{shell,ingested} = \frac{DM_{shell,sample}}{FM_{food,sample}} \times FM_{food} . \qquad (2)$$

$DM_{shell,sample}$ was calculated as:

$$DM_{shell,sample} = \sum_{i=1}^{m} \left(\left(\frac{DM_{shell,size\ i,subsample}}{N_{size\ i,subsample}} \right) \times N_{size\ i,sample} \right), \qquad (3)$$

where $DM_{shell,size\ i,subsample}$ and $N_{size\ I,subsample}$ are the total dry shell mass and the total number of *P. laevis* size class i in a subsample of each prey sample, and $N_{size\ i,sample}$ is the number of *P. laevis* of size class i in each prey sample. The flesh mass of food ingested, FM_{food}, was calculated as:

$$FM_{food} = \left(FM_{given\ food} \times \left(\frac{N_{sample}}{FM_{sample}} \right) - N_{retained\ food} \right) \times \frac{FM_{sample}}{N_{sample}}, \qquad (4)$$

where $FM_{given\ food}$ is the flesh mass of the food given to each experimental bird in a trial, N_{sample} is the number of *P. laevis* in each prey sample, and $N_{retained\ food}$ is the number of retained food items in the same trial. We used r to correct dry mass of ingested shell ($DM_{shell,ingested}$) from dry mass of droppings retained on the 0.3 mm mesh ($DM_{droppings}$) for the dropping samples collected in the field.

We calculated the shell processing rate (SPR) of red knots in Bohai Bay as follows:

$$SPR = \frac{DM_{shell,ingested}}{N_{droppings}} \times dropping\ interval^{-1} , \qquad (5)$$

where N_{drop} is the total number of droppings in each sample (60 droppings per sample).

To check the accuracy of shell size reconstruction from dropping analyses, the size distribution of *P. laevis* in droppings retained on the 0.3 mm mesh and in the food presented was compared. The size distribution of *P. laevis* in dropping samples was determined based on the measurement of unbroken hinges in the subsamples. Using the relationship between shell length and hinge and top height (see Table 1), the size distribution of *P. laevis* in droppings remaining on the mesh was calculated.

We tested for covariation between the dry mass of droppings before and after being sieved with the 0.3 mm mesh, and the variance in r among individuals, using GLM. The intercept of the regression represents the fact that at the end of the trials red knots had not fully emptied their guts, assuming no interaction between the amount ingested and what was left in the gut at the end of the experiment. We tested dropping intervals for significant variance among date and subspecies using ANCOVA. We also tested the difference of size distribution of *P. laevis* in food presented and ingested (dropping fragments remaining on the 0.3 mm mesh) using Chi-square tests. All analyses were conducted using SYSTAT13 (SYSTAT Software Inc.).

3. What makes *P. laevis* so easy to digest?

How can red knots with such small gizzards have such high processing rates? Given that the ratio between the amount of shell mass ingested and eventually retained on a 0.3 mm mesh, the correction factor r is close to 1 for red knots feeding on the bivalves *Macoma balthica* and *Cerastoderma edule* in the Wadden Sea (Dekinga and Piersma, 1993), and 1.5 for red knots feeding on the bivalves *Dosinia isocardia* and *Loripes lucinalis* at Banc d'Arguin, Mauritania (Onrust et al., 2013), a ratio of 2.3 suggests that *P. laevis* is more readily crushed to fine fragments (of the kind that fall through the mesh). By making a comparison with other higher quality mollusc species, we here examined the possibility that the forces required to crush ingested bivalves explain why *P. laevis* is so easy to process despite the unfavourable flesh to shell mass ratio (see Fig. 2).

To compare the breaking force of *P. laevis* (from China), *C. edule* and *M. balthica* (from the Dutch Wadden Sea), and *D. isocardia* and *L. lucinalis* (from Banc d'Arguin, Mauritania), shell strength was determined using a device built and described by Buschbaum and coleagues (Buschbaum et al., 2007). Individual, alcohol-preserved intact bivalves of a variety of sizes were mounted on a load table, after which pressure was applied by the upper plate at constant speed ($0.5\,cm\,s^{-1}$) until the shells crushed. At this point the conjoint ammeter recorded the highest voltage, which was transformed into a force measure (N). The maximum force required to crush a shell was recorded to the nearest 0.1 N. Tests showed that alcohol-stored bivalves require the same forces to crush as freshly collected ones (T. Oudman, personal communication). We tested for correlations between the ratio of dry mass of ingested shell to dry mass of droppings retained on 0.3 mm mesh and size-corrected shell breaking forces with Spearman's rank test using *R* (R Development Core Team, 2011).

Table 2. Average density of harvestable molluscs in mudflats and their biomass, and percentages of harvestable molluscs in mudflats and in diet in 2008

Species name	Family	Molluscs in mudflats			Molluscs in diet		E
		Average density (individuals m^{-2})	Biomass (g AFDM m^{-2})	% Biomass	Biomass (mg AFDM dropping^{-1})	% Biomass	
Potamocorbula laevis	Corbulidae	15,896	3.13±2.3	69.18	19.02±6.82	92.74	0.01
Moerella iridescens	Tellinidae	34	0.18±0.29	3.98	0.92±1.03	4.49	−0.08
Mactra quadrangularis	Mactridae	12	0.12±0.28	2.65	0.39±0.31	1.90	−0.29
Theora fragilis	Semelidae	16	0.003±0.005	0.07	0.12±0.18	0.59	0.74
Musculus senhousei	Mytilidae	5	0.001±0.001	0.02	0.03±0.08	0.15	0.67
Stenothyra glabra	Stenothyridae	31	0.36±0.68	7.96	0.02±0.04	0.10	
Cyclina sinensis	Veneridae	–	–	–	0.005±0.01	0.02	1.00
Nassarius variciferus	Nassariidae	0.8	0.36±1.09	7.96	0.003±0.004	0.01	
Natica janthostomoides	Naticidae	1.6	0.19±0.58	4.20	0	0	
Mitrella bella	Columbellidae	0.8	0.16±0.48	3.54	0	0	
Assiminea latericea	Assimineidae	3	0.02±0.05	0.44	0	0	
Lingula unguis*	Lingulidae	0.8	0.0004±0.001	0.01	0	0	

*Lingula unguis belongs to Inarticulata of Atremata.

For average density of molluscs in mudflats, *N*=72 cores.

Biomass in mudflats and in diet is expressed as g flesh ash-free dry mass per m^2 (*N*=72 cores) and mg flesh ash-free dry mass per dropping (*N*=540 droppings), respectively (means ± s.e.m.).

E, preference index calculated for red knots for the six known bivalve prey.

RESULTS AND DISCUSSION
1. Testing the predictions

Red knots fed on eight of the 12 available mollusc species on the intertidal mudflats (Table 2). Bivalves accounted for 99% of the total ingested biomass in the diet, with *P. laevis* being by far the most important prey. However, when only considering the available bivalves in the mudflats, the Ivlev selection index and Chi-square tests for biomass values showed that red knots took *P. laevis* in proportion rather than selecting them (E=0.01; χ^2=6.46, d.f.=4, *P*=0.17; Fig. 1). In northern Bohai Bay, the density of *P. laevis* in the mudflats was much higher than that of other molluscs (Table 2), and in fact much exceeded that of the mollusc prey fed upon by red knots in northwest Europe and Latin America (Zwarts et al., 1992; González et al., 1996; Bocher et al., 2007). However, the quality of 1–7 mm long *P. laevis* measured in the traditional way (energy content divided by ballast shell mass) was only 1.03–1.68 kJ g^{-1} DM$_{shell}$ (*N*=2795); the quality of the most frequently eaten length class (3 mm) was 1.32 kJ g^{-1} DM$_{shell}$ (Fig. 2). This is only about a third of the predicted value of 3.5–4.5 kJ g^{-1} DM$_{shell}$ needed by birds to maintain a stable gizzard mass (5.94 g) between departure from northwest Australia and arrival in China (Battley et al., 2005). Furthermore, it is lower than the quality of prey recorded at three main stopovers and three main wintering areas in four other subspecies (van Gils et al., 2005a).

The gizzards of red knots in Bohai Bay weighed on average 4.9±0.8 g (mean ± s.e.m., range 3.1–6.7 g, *N*=27 birds) (Fig. 3). This is notably smaller than that recorded at the wintering areas (5.9 g for *C. c. piersmai* in NW Australia and 9.3 g for *C. c. rogersi* in New Zealand) (van Gils et al., 2005a). The *C. c. piersmai* knots thus showed a 17% reduction in gizzard mass between NW Australia and Bohai Bay, which is similar to the reported loss in mass of 18% in great knot *Calidris tenuirostris* between the start and end of the migration from NW Australia to China (Battley et al., 2001).

On the basis of the measured prey quality of 1.32 kJ g^{-1} DM$_{shell}$ (for 3 mm long *P. laevis*), and the measured gizzard mass of 4.9 g, the digestive rate model used by Battley and colleagues (Battley et al., 2005) would predict an assimilated intake rate of 2.7 J s^{-1}. This is much lower than the estimated requirement of 3.6 J s^{-1} [using

information in van Gils et al. (van Gils et al., 2005a)], if red knots were to double arrival body mass in the course of 29 days. Clearly, something is missing in our argument.
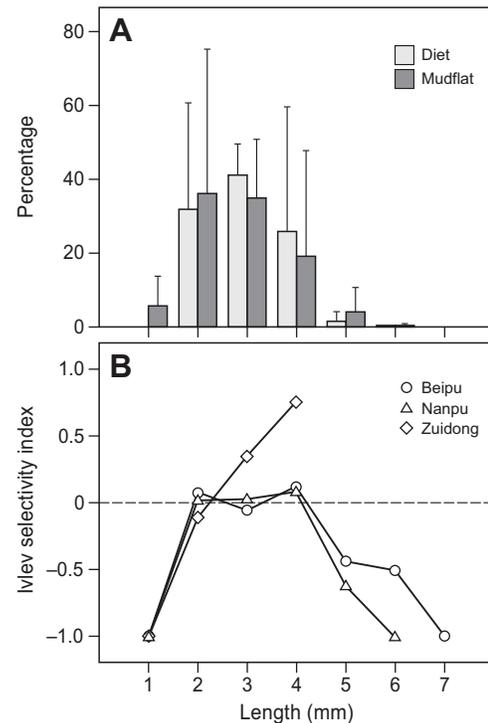

Fig. 1. (A) Percentage of all size classes of total *Potamocorbula laevis* individuals available in mudflats and the relative frequency of all size classes of ingested *P. laevis* by red knots at Beipu, Nanpu and Zuidong (means ± s.d., *N*=3 sites, including 20,438 *P. laevis* in benthos samples and 4497 *P. laevis* in droppings samples). (B) Diet selectivity of size classes of available *P. laevis* based on dropping analysis. Among the size classes of ingested *P. laevis* corrected by factors derived from the digestion experiment (2–4 mm; see section 2, 'Energy intake rates', Materials and methods), there is only a clear preference for 3 and 4 mm long *P. laevis* (Ivlev index>0) at Zuidong.
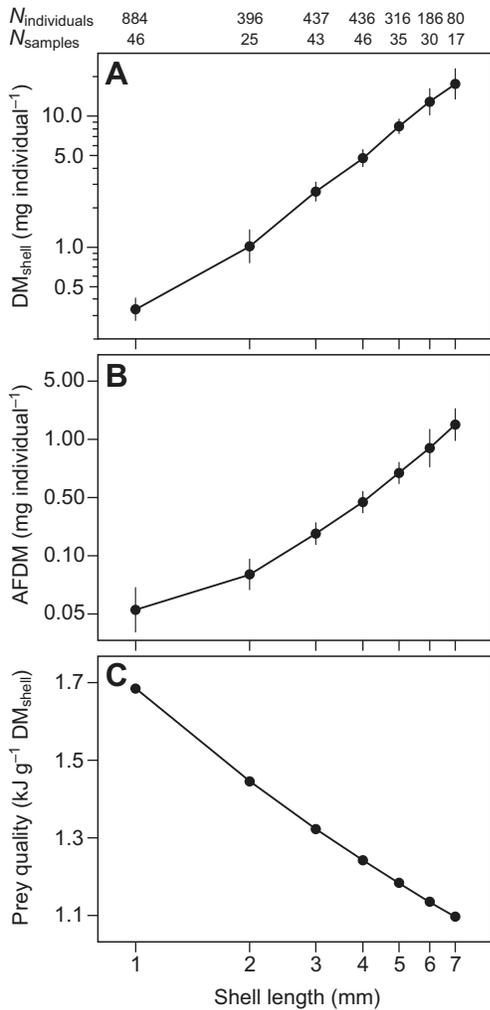
$N_{individuals}$ 884   396   437   436 316 186 80
$N_{samples}$ 46   25   43   46   35 30 17

Fig. 2. The relationship between shell length and dry shell mass (DM_{shell}; A, mean ± s.d.), ash-free dry mass ($AFDM_{flesh}$; B, mean ± SD) and predicted prey quality (C) of available *P. laevis* in mudflats.

2. Energy intake rates

The median of the dropping intervals was 79 s dropping^{-1} (N=149 dropping intervals; range 48–127 s; Fig. 4). We detected no effect of subspecies (P=0.95), or date as a linear covariate (P=0.57), on dropping interval. The dropping interval of red knots in Bohai Bay was similar to that of birds in the Wadden Sea, NW Europe (80 s)

(Zwarts and Blomert, 1992), but shorter than that of birds in San Antonio Oeste, Argentina (143 s) (González et al., 1996).

The regression of the mass of shell fragments (retained on a 0.3 mm mesh) on the ingested shell mass per trial was significant (R^2=0.86, P<0.001) (Fig. 5). The slope of the regression (i.e. *r*) (Dekinga and Piersma, 1983) was estimated at 2.3. Therefore, the estimate of $DM_{shell,ingested}$ of *P. laevis* had to be corrected by multiplying $DM_{droppings}$ by 2.3, and indicated that 57% of ingested shell material was lost through the 0.3 mm mesh.

The size distributions of *P. laevis* eaten and excreted as droppings (retained on a mesh of 0.3 mm) were significantly different (χ^2=4.27, d.f.=1, P=0.043). We thus had to correct for bias in estimated ingested shell size by multiplying the number of a certain size of *P. laevis* in droppings maintained on the mesh with the ratio of the relative frequency in the food eaten to the relative frequency retrieved from the dropping samples of a certain size. For the three relevant size classes the correction factors were, respectively, 2.67 (2 mm), 0.60 (3 mm) and 1.35 (4 mm).

At an average shell length of ingested *P. laevis* of 3 mm, the shell processing rate of red knots was calculated as 3.93 mg s^{-1} (Fig. 6). This is higher than predicted for red knots with a gizzard mass of 4.9 g (i.e. 1.2 mg s^{-1}) (van Gils et al., 2003) and is even higher than the predicted value for knots with large gizzards (2.58 mg s^{-1}) (van Gils et al., 2003). Adding a few more robust assumptions to the calculation (on energy density and digestive efficiency, see Appendix), the metabolizable energy intake rate was estimated at 5.1 J s^{-1}, a value that is higher than the predicted minimum energy intake rate of red knots staging in Bohai Bay (3.6 J s^{-1}). A metabolizable energy intake rate of *ca.* 5 J s^{-1} is similar to the values for red knots in the Dutch Wadden Sea: 5.5 J s^{-1} (on a diet of *M. balthica*) (Zwarts and Blomert, 1992; Piersma et al., 1994) and higher than 3.6 J s^{-1} for red knots eating a mixed diet that included low quality snails *Hydrobia ulvae* (van Gils et al., 2003). It is also similar to an estimate for red knots feeding on small mussels *Brachidontes* in Argentina (4.6 J s^{-1}) (González et al., 1996; van Gils et al., 2005a). All of this indicates that *P. laevis* have characteristics that make them easy to process in the digestive tract.

3. What makes *P. laevis* so easy to digest?

The breaking force of the five bivalve prey of red knots increased with length (Fig. 7): small bivalves were easiest to crush. Although *P. laevis* was not the easiest to crush among the bivalves tested here, their small size ensured that the breaking force of prey eaten by red knots in Bohai Bay was less than in other previously studied foraging contexts (Dekinga and Piersma, 1993; Onrust et al., 2013). As predicted, there was a significant negative relationship between
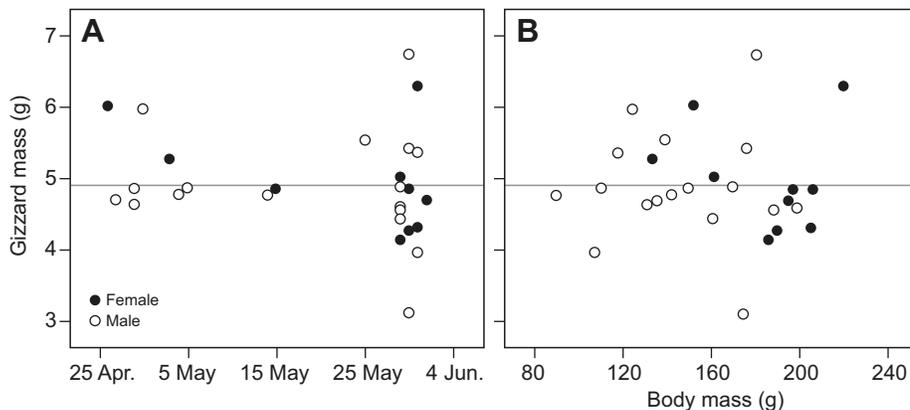

Fig. 3. The gizzard mass of red knots against date and body mass (N=27). The solid line is the mean gizzard mass (4.9 g). There are no correlations between date, body mass and sex and gizzard mass (P=0.29, P=0.69, P=0.91, respectively).
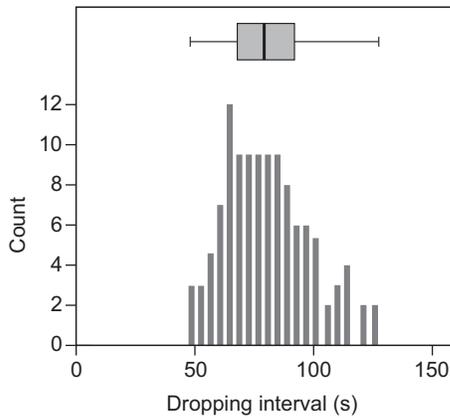
Fig. 4. Frequency distribution (and box plot including median, and 50% and full ranges) of observed dropping intervals of red knots on the Bohai mudflats. The median of 79 s is similar to the mean of 81 s.

the (ingested size-specific) ratio r and measured breaking force (Spearman's rank test, $\rho=-0.97$, $P=0.005$; Fig. 7).

General discussion

According to Zwarts and Blomert (Zwarts and Blomert, 1992), small prey with low flesh to shell mass ratios are generally unprofitable to red knots. Our trail of discovery has led us to conclude that when small prey occur in as a high density as is the case with the freshly settled *P. laevis* in Bohai Bay (over $10,000 \, m^{-2}$), their statement is not true. We also found that the quantification of prey quality in molluscivores has missed the element of breaking force. However, before examining issues of symmorphosis, we will re-examine the functional rule that best explains the diet and feeding behaviour of red knots staging in Bohai Bay.

In trying to explain diet selection under the assumption that organisms will maximize energy intake rate per unit time (MacArthur and Pianka, 1966; Pulliam, 1974; Charnov, 1976), the classic
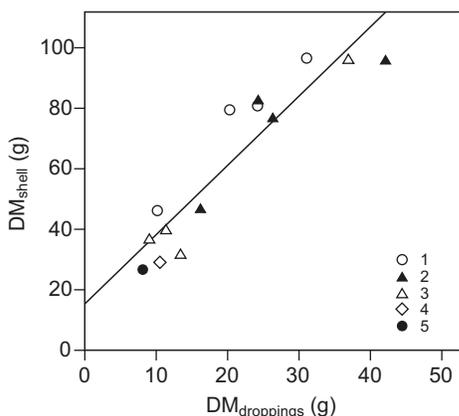

Fig. 5. Dry mass of ingested shell of *P. laevis* by red knots as a function of dry mass of red knot droppings (DM$_{droppings}$) retained on a 0.3 mm mesh. The linear regression line represents the following equation: $y=2.3x+14.8$ ($R^2=0.86$, $P<0.001$, $N=14$). Symbols represent different individuals (1–5) in the experiment. The intercept is greater than zero as red knots did not have time to empty their gizzards as the beginning of the trials. There is variation in the ratio, r, of dry mass of ingested shell to dry mass of droppings retained on the 0.3 mm mesh among individuals but it is not significant ($P=0.09$).
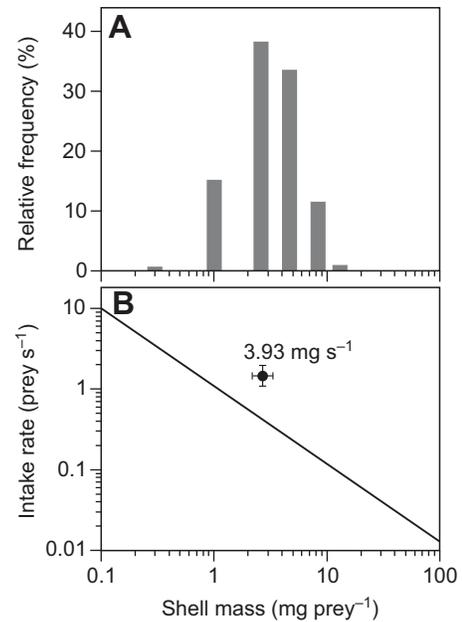

Fig. 6. Relative frequency distribution (A) of shell masses of available *P. laevis* at the Bohai study site ($N=20,438$) and the intake rates of red knots as a function of shell mass (B). In B, the solid line (from van Gils et al., 2003) gives the general linear regression of intake rates on individual prey shell mass based on van Gils and colleagues' experimental observations. The dot presents the data point for ingested *P. laevis* (3 mm) in Bohai Bay. The horizontal s.e.m. bar of the dot is the standard error of mean shell mass of 3 mm long *P. laevis* (2.7 ± 0.5 mg, mean $\pm$ s.e.m., $N=43$) and the vertical s.e.m. bar of the dot is the standard error of mean numerical intake rate (1.5 ± 0.4 prey s^{-1}, mean $\pm$ s.e.m., $N=9$).

representation has been the contingency model (CM), which predicts that intake rates will be constrained by search and handling time only (Belovsky, 1984; Stephens and Krebs, 1986). The DRM was developed for predators feeding on prey with indigestible bulk, and predicts that energy intake rate of predators depends on the digestive rate, which makes processing time rather than search/handling time the limiting factor (Verlinden and Wiley, 1989; Hirakawa, 1995; van Gils et al., 2005b). The subjects of the present study are well-known digestively constrained predators limited by the amount of shell mass that can be processed per unit time (Piersma et al., 1993a; van Gils et al., 2003; van Gils et al., 2005b). Here, we have shown that because the small *P. laevis* were so easy to crush, high processing rates were possible. Given the high densities in which *P. laevis* occur on the Bohai mudflats (Table 2) (H.-Y.Y. et al., unpublished data), search times were unlikely to be limiting, but it remains possible that the energy intake rates were constrained by handling rather than internal shell digestion times (see van Gils et al., 2003). To verify the idea that red knots were limited by handling time (and thus obey the CM; this is when the time between two prey ingestions would equal handling time) (Stephens and Krebs, 1986), on the basis of bivalve size (Piersma et al., 1995), we predicted the handling time of 3 mm long *P. laevis* as 0.2 s. Our empirical assessment of the interval between ingestions is based on the shell mass per individual prey item divided by shell processing rate (section 2, 'Energy intake rates', Materials and methods), and equals 0.7 s. Clearly, in red knots eating small *P. laevis* in Bohai Bay, estimated handling time is much shorter than the estimated average interval between two prey ingestions (0.7 s), and we conclude that energy intake rates were not constrained by handling time.
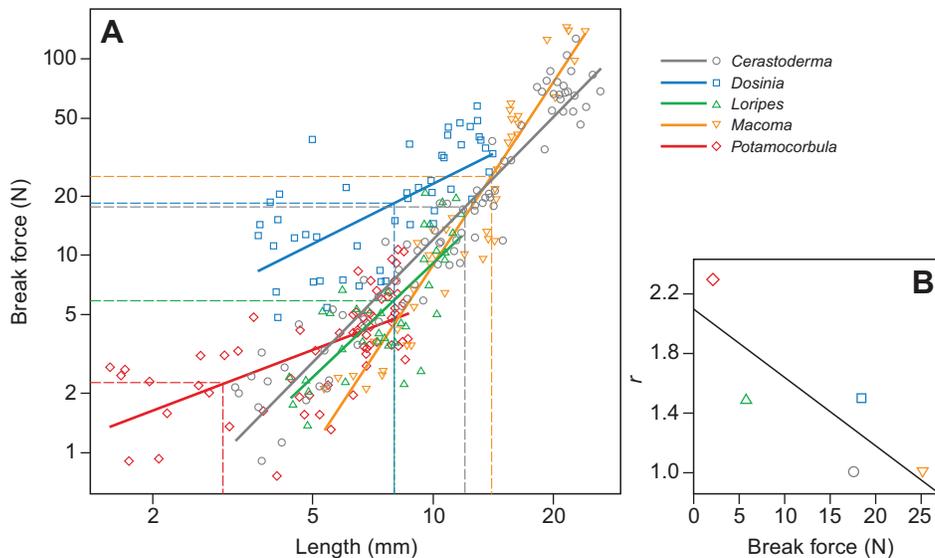
Fig. 7. (A) Breaking force as a function of individual shell length in five bivalve species: *Cerastoderma edule* (3–25 mm, N=71) and *Macoma balthica* (5–24 mm, N=52) (two of the main prey of red knots in the Wadden Sea), *Dosinia isocardia* (3–14 mm, N=53) and *Loripes lucinalis* (4–11 mm, N=40) (two of the main prey of red knots in Banc d'Arguin), and *P. laevis* (1–8 mm, N=69) in Bohai Bay. The dashed lines represent breaking force of the five species for the shell length most frequently eaten in the field. (B) Relationship between shell breaking force of the five species at the shell length that red knots eat most often and the ratio (r) of dry mass of ingested shell to dry mass of droppings retained on a 0.3 mm mesh of the five species (R^2=0.59, $F_{1,3}$=6.72, P=0.08).

On the basis of the existing version of the DRM (van Gils et al., 2003), which uses gizzard mass and an individual prey's shell mass as input, the predicted time between two prey ingestions of red knots feeding on small *P. laevis* should be 2.2 s rather than 0.7 s. Clearly, the existing version of the DRM that uses prey quality expressed as flesh to shell mass ratios (van Gils et al., 2003; van Gils et al., 2005a) does not capture the feeding of red knots in Bohai Bay well. This is because prey size was so much smaller here than the molluscs that had previously featured in red knot diet studies, and because the forces needed to crush tiny prey are so small (Fig. 7). That small crushable prey enable high shell processing rates was in hindsight already clear from a data point for red knots experimentally fed small *Macoma balthica* [see fig. 3 of van Gils et al. (van Gils et al., 2003)], showing far higher processing rates than predicted for birds with small gizzards. In fact, small *M. balthica* turn out to be the most crushable among all bivalves measured (Fig. 7A). Small prey with low flesh to shell mass ratios were considered unprofitable, but no studies so far have incorporated abundantly available small food items made profitable by their high crushability. We conclude that the DRM for molluscivores requires revision. Proper weight should be given to size-dependent crushing force in addition to relative shell mass. This exercise is beyond the scope of the present paper (T. Oudman, H.-Y.Y., J.A.v.G. and T.P., in preparation).

Unexpected feeding conditions (high densities of small crushable prey with low flesh to shell mass ratios) at the Yellow Sea staging site of red knots caused the predictions by Battley and colleagues (Battley et al., 2005), which we thought were quite robust, to be rejected on the basis of the empirical scrutiny presented here. In fact, the food conditions in Bohai Bay were so far outside the previously described range (van Gils et al., 2005a) that we have here presented a new combination of phenotypes and environments. This indicates that the phenotype of these migrants is even more flexible than we thought (Piersma and van Gils, 2011).

Clearly, selection pressures to make the most of the environmental context encountered, in this case to raise to the challenge of refuelling as fast and as economically as possible, leads to reductions in the digestive apparatus so that wastage of capacity is reduced. This yields the general point that for metabolic machinery, at least in time-stressed migrating birds, safety factors (Diamond, 1993; Diamond, 2002) are kept small. Thus, the red knots staging in Bohai Bay, China, in comparison

with red knots elsewhere in the world, provide a truly elegant example of the principle of symmorphosis (Taylor and Weibel, 1981; Weibel, 2000).

APPENDIX

Energy intake rate (EIR) of the two red knot subspecies in Bohai Bay was calculated in a series of steps as follows:

$$\mathrm{DM_{shell,ingested}} = r \times \mathrm{DM_{droppings}}, \qquad (A1)$$

$$\mathrm{AFDM_{ingested}} = \mathrm{DM_{shell,ingested}} \times \alpha, \qquad (A2)$$

where dry mass is in g and α is the ratio of ash free dry mass of flesh to dry shell mass of ingested *P. laevis* at the average shell length (0.0741, 3 mm, N=437).

$$\mathrm{Biomass\ equivalent} = \mathrm{AFDM_{ingested}} / N_{drop}, \qquad (A3)$$

where biomass equivalent is g AFDM dropping^{-1} and dry mass is in g;

$$\mathrm{Biomass\ intake\ rate} = \frac{\mathrm{biomass\ equivalent}}{\mathrm{dropping\ interval}}, \qquad (A4)$$

where biomass intake rate is g AFDM s^{-1} and dropping interval is in s;

$$\mathrm{Gross\ EIR} = \mathrm{biomass\ intake\ rate} \times d \times 1000, \qquad (A5)$$

where EIR is in J s^{-1}, d is in kJ g^{-1} AFDM; and

$$\mathrm{Metabolizable\ EIR} = \mathrm{gross\ EIR} \times a. \qquad (A6)$$

The biomass equivalent calculated is 23.0±5.9 mg AFDM dropping^{-1} and biomass intake rate is 0.291±0.075 mg AFDM s^{-1}.

REFERENCES

Atkinson, P. W., Baker, A. J., Bennett, K. A., Clark, N. A., Clark, J. A., Cole, K. B., Dekinga, A., Dey, A., Gillings, S., Gonzalez, P. M. et al. (2007). Rates of mass gain and energy deposition in red knot on their final spring staging site is both time- and condition-dependent. *J. Appl. Ecol.* **44**, 885-895.

Baker, A. J., Gonzalez, P. M., Piersma, T. and Niles, L. J., de Lima, S., do Nascimento, I., Atkinson, P. W., Collins, P., Clark, N. A., Minton et al. (2004). Rapid population decline in red knots: fitness consequences of decreased refuelling rates and late arrival in Delaware Bay. *Proc. R. Soc. B*, **271**, 875-882.

Battley, P. F. and Piersma, T. (2005). Adaptive interplay between feeding ecology and features of the digestive tract in birds. In *Physiological and Ecological Adaptations to Feeding in Vertebrates* (ed. J. M. Starck and T. Wang), pp. 201-228. Enfield, NH: Science Publishers.

Battley, P. F., Piersma, T., Dietz, M. W., Tang, S. X., Dekinga, A. and Hulsman, K. (2000). Empirical evidence for differential organ reductions during trans-oceanic bird flight. *Proc. Biol. Sci.* **267**, 191-195.

Battley, P. F., Dekinga, A., Dietz, M. W., Piersma, T., Tang, S. and Hulsman, K. (2001). Basal metabolic rate declines during long-distance migratory flight in great knots. *Condor* **103**, 838-845.

Battley, P. F., Rogers, D. I., van Gils, J. A., Piersma, T., Hassell, C. J., Boyle, A. and Yang, H. Y. (2005). How do red knots *Calidris canutus* leave Northwest Australia in May and reach the breeding grounds in June? Predictions of stopover times, fuelling rates and prey quality in the Yellow Sea. *J. Avian Biol.* **36**, 494-500.

Belovsky, G. E. (1984). Herbivore optimal foraging – a comparative test of 3 models. *Am. Nat.* **124**, 97-115.

Bocher, P., Piersma, T., Dekinga, A., Kraan, C., Yates, M. G., Guyot, T., Folmer, E. O. and Radenac, G. (2007). Site- and species-specific distribution patterns of molluscs at five intertidal soft-sediment areas in northwest Europe during a single winter. *Mar. Biol.* **151**, 577-594.

Buschbaum, C., Buschbaum, G., Schrey, I. and Thieltges, D. (2007). Shell boring polychaetes affect gastropod shell strength and crab predation. *Mar. Ecol. Prog. Ser.* **329**, 123-130.

Charnov, E. L. (1976). Optimal foraging: attack strategy of a mantid. *Am. Nat.* **110**, 141-151.

Choi, C. Y., Gan, X. J., Ma, Q., Zhang, K. J., Chen, J. K. and Ma, Z. J. (2009). Body condition and fuel deposition patterns of calidrid sandpipers during migratory stopover. *Ardea* **97**, 61-70.

Dekinga, A. and Piersma, T. (1983). Reconstructing diet composition on the basis of faeces in a mollusc-eating wader, the knot *Calidris canutus*. *Bird Study* **40**, 144-156.

Dekinga, A., Dietz, M. W., Koolhaas, A. and Piersma, T. (2001). Time course and reversibility of changes in the gizzards of red knots alternately eating hard and soft food. *J. Exp. Biol.* **204**, 2167-2173.

Diamond, J. (1993). Evolutionary physiology. In *The Logic of Life. The Challenge of Integrative Physiology* (ed. C. A. R. Boyd and D. Noble), pp. 89-111. Oxford: Oxford University Press.

Diamond, J. (2002). Quantitative evolutionary design. *J. Physiol.* **542**, 337-345.

Dietz, M. W., Dekinga, A., Piersma, T. and Verhulst, S. (1999). Estimating organ size in small migrating shorebirds with ultrasonography: An intercalibration exercise. *Physiol. Biochem. Zool.* **72**, 28-37.

González, P. M., Piersma, T. and Verkuil, Y. (1996). Food, feeding, and refuelling of red knots during northward migration at San Antonio Oeste, Rio Negro, Argentina. *J. Field Ornithol.* **67**, 575-591.

Hassell, C. J., Southey, I., Boyle, A. and Yang, H. Y. (2011). Red knot *Calidris canutus*: subspecies and migration in the East Asian-Australasian flyway – where do all the red knot go? *Birding Asia* **16**, 89-93.

Hirakawa, H. (1995). Diet optimization with a nutrient or toxin constraint. *Theor. Popul. Biol.* **47**, 331-346.

Hua, N., Piersma, T. and Ma, Z. (2013). Three-phase fuel deposition in a long-distance migrant, the red knot (*Calidris canutus piersmai*), before the flight to high Arctic breeding grounds. *PLoS ONE* **8**, e62551.

Jacobs, J. (1974). Quantitative measurement of food selection: a modification of the forage ratio and Ivlev's electivity index. *Oecologia* **14**, 413-417.

Kersten, M. and Piersma, T. (1987). High levels of energy expenditure in shorebirds; metabolic adaptations to an energetically expensive way of life. *Ardea* **75**, 175-187.

Kraan, C., van Gils, J. A., Spaans, B., Dekinga, A., Bijleveld, A. I., van Roomen, M., Kleefstra, R. and Piersma, T. (2009). Landscape-scale experiment demonstrates that Wadden Sea intertidal flats are used to capacity by molluscivore migrant shorebirds. *J. Anim. Ecol.* **78**, 1259-1268.

Kraan, C., van Gils, J. A., Spaans, B., Dekinga, A. and Piersma, T. (2010). Why Afro-Siberian red knots *Calidris canutus canutus* have stopped staging in the western Dutch Wadden Sea during southward migration. *Ardea* **98**, 155-160.

MacArthur, R. H. and Pianka, E. R. (1966). On the optimal use of a patchy environment. *Am. Nat.* **100**, 603-609.

McWilliams, S. R. and Karasov, W. H. (2001). Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. *Comp. Biochem. Physiol.* **128A**, 577-591.

McWilliams, S. R., Guglielmo, C., Pierce, B. and Klaassen, M. (2004). Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. *J. Avian Biol.* **35**, 377-393.

Morrison, R. I. G., Davidson, N. C. and Wilson, J. R. (2007). Survival of the fattest: body stores on migration and survival in red knots *Calidris canutus islandica*. *J. Avian Biol.* **38**, 479-487.

Onrust, J., de Fouw, J., Oudman, T., van der Geest, M., Piersma, T. and van Gils, J. A. (2013). Red knot diet reconstruction revisited: context dependence revealed by experiments at Banc d'Arguin, Mauritania. *Bird Study* doi:10.1080/00063657.2013.811213.

Piersma, T. (1994). Close to the edge: energetic bottlenecks and the evolution of migratory pathways in knots. PhD thesis, University of Groningen, The Netherlands.

Piersma, T. (2007). Using the power of comparison to explain habitat use and migration strategies of shorebirds worldwide. *J. Ornithol.* **148 Suppl.**, 45-59.

Piersma, T. and Lindström, Å. (1997). Rapid reversible changes in organ size as a component of adaptive behaviour. *Trends Ecol. Evol.* **12**, 134-138.

Piersma, T. and van Gils, J. A. (2011). *The Flexible Phenotype: a Body-Centred Integration of Ecology, Physiology, and Behaviour.* Oxford: Oxford University Press.

Piersma, T., Koolhaas, A. and Dekinga, A. (1993a). Interactions between stomach structure and diet choice in shorebirds. *Auk* **110**, 552-564.

Piersma, T., Hoekstra, R., Dekinga, A., Koolhaas, A., Wolf, P., Battley, P. and Wiersma, P. (1993b). Scale and intensity of intertidal habitat use by knots *Calidris canutus* in the western Wadden Sea in relation to food, friends and foes. *Neth. J. Sea Res.* **31**, 331-357.

Piersma, T., Verkuil, Y. and Tulp, I. (1994). Resources for long-distance migration of knots *Calidris canutus islandica* and *C. c. canutus*: how broad is the temporal exploitation window of benthic prey in the western and eastern Wadden Sea? *Oikos* **71**, 393-407.

Piersma, T., van Gils, J., de Goeij, P. and van der Meer, J. (1995). Holling's functional response model as a tool to link the food-finding mechanism of a probing shorebird with its spatial distribution. *J. Anim. Ecol.* **64**, 493-504.

Piersma, T., Gudmundsson, G. A., Davidson, N. C. and Morrison, R. I. G. (1996). Do arctic-breeding red knots (*Calidris canutus*) accumulate skeletal calcium before egg laying? *Can. J. Zool.* **74**, 2257-2261.

Piersma, T., Dietz, M. W., Dekinga, A., Nebel, S., van Gils, J., Battley, P. F. and Spaans, B. (1999a). Reversible size-changes in stomachs of shorebirds: when, to what extent, and why? *Acta Ornithologica* **34**, 175-181.

Piersma, T., Gudmundsson, G. A. and Lilliendahl, K. (1999b). Rapid changes in the size of different functional organ and muscle groups during refueling in a long-distance migrating shorebird. *Physiol. Biochem. Zool.* **72**, 405-415.

Piersma, T., Gessaman, J. A., Dekinga, A. and Visser, G. H. (2004). Gizzard and other lean components increase, yet Basal Metabolic Rates decrease, when red knots Calidris canutus are shifted from soft to hard-shelled food. *J. Avian Biol.* **35**, 99-104.

Piersma, T., Rogers, D. I., Gonzalez, P., Zwarts, L., Niles, L. J., de Lima Serrano do Nascimento, I., Minton, C. D. T. and Baker, A. J. (2005). Fuel storage rates before northward flights in red knots worldwide. In *Birds of Two Worlds: The Ecology and Evolution of Migratory Birds* (ed. R. Greenberg and P. Marra), pp. 262-274. Baltimore, MD: Johns Hopkins University Press.

Pulliam, H. R. (1974). On the theory of optimal diets. *Am. Nat.* **108**, 59-74.

R Development Core Team (2011). A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available at: http://www.R-project.org.

Rogers, D. I., Yang, H. Y., Hassell, C. J., Boyle, A. N., Rogers, K. G., Chen, B., Zhang, Z. W. and Piersma, T. (2010). Red knots (*Calidris canutus piersmai* and *C. c. rogersi*) depend on a small threatened staging area in Bohai Bay, China. *Emu* **110**, 307-315.

Stephens, D. W. and Krebs, J. R. (1986). *Foraging Theory.* Princeton, NJ: Princeton University Press.

Taylor, C. R. and Weibel, E. R. (1981). Design of the mammalian respiratory system. I. Problem and strategy. *Respir. Physiol.* **44**, 1-10.

van Gils, J. A., Piersma, T., Dekinga, A. and Dietz, M. W. (2003). Cost-benefit analysis of mollusc-eating in a shorebird. II. Optimizing gizzard size in the face of seasonal demands. *J. Exp. Biol.* **206**, 3369-3380.

van Gils, J. A., Battley, P. F., Piersma, T. and Drent, R. (2005a). Reinterpretation of gizzard sizes of red knots world-wide emphasises overriding importance of prey quality at migratory stopover sites. *Proc. Biol. Sci.* **272**, 2609-2618.

van Gils, J. A., de Rooij, S. R., van Belle, J., van der Meer, J., Dekinga, A., Piersma, T. and Drent, R. (2005b). Digestive bottleneck affects foraging decisions in red knots *Calidris canutus*. I. Prey choice. *J. Anim. Ecol.* **74**, 105-119.

van Gils, J. A., Dekinga, A., Spaans, B., Vahl, W. K. and Piersma, T. (2005c). Digestive bottleneck affects foraging decisions in red knots *Calidris canutus*. II. Patch choice and length of working day. *J. Anim. Ecol.* **74**, 120-130.

van Gils, J. A., Piersma, T., Dekinga, A. and Battley, P. F. (2006a). Modelling phenotypic flexibility: an optimality analysis of gizzard size in red knots (*Calidris canutus*). *Ardea* **94**, 409-420.

van Gils, J. A., Spaans, B., Dekinga, A. and Piersma, T. (2006b). Foraging in a tidally structured environment by red knots (*Calidris canutus*): ideal, but not free. *Ecology* **87**, 1189-1202.

Verkuil, Y. I., Karlionova, N., Rakhimberdiev, E., Jukema, J., Wijmenga, J. J., Hooijmeijer, J. C. E. W., Pinchuk, P., Wymenga, E., Baker, A. J. and Piersma, T. (2012). Losing a staging area: eastward redistribution of Afro-Eurasian ruffs is

associated with deteriorating fuelling conditions along the western flyway. *Biol. Conserv.* **149**, 51-59.

Verlinden, C. and Wiley, R. H. (1989). The constraints of digestive rate: an alternative model of diet selection. *Evol. Ecol.* **3**, 264-273.

Weibel, E. R. (2000). *Symmorphosis. On Form and Function in Shaping Life.* Cambridge, MA: Harvard University Press.

Yang, H.-Y., Chen, B., Barter, M., Piersma, T., Zhou, C.-F., Li, F.-S. and Zhang, Z.-W. (2011). Impacts of tidal land reclamation in Bohai Bay, China: ongoing losses of critical Yellow Sea waterbird staging and wintering sites. *Bird Conserv. Int.* **21**, 241-259.

Zwarts, L. and Blomert, A.-M. (1992). Why knots *Calidris canutus* take medium-sized *Macoma balthica* when six prey species are available. *Mar. Ecol. Prog. Ser.* **83**, 113-128.

Zwarts, L. and Wanink, J. H. (1993). How the food supply harvestable by waders in the Wadden Sea depends on the variation in energy density, body weight, biomass, burying depth and behaviour of tidal-flat invertebrates. *Neth. J. Sea Res.* **31**, 441-476.

Zwarts, L., Ens, B. J., Kersten, M. and Piersma, T. (1990). Moult, mass and flight range of waders ready to take off for long-distance migrations. *Ardea* **78**, 339-364.

Zwarts, L., Blomert, A.-M. and Wanink, J. H. (1992). Annual and seasonal variation in the food supply harvestable by knot *Calidris canutus* staging in the Wadden Sea in late summer. *Mar. Ecol. Prog. Ser.* **83**, 129-139.

Zwarts, L., Ens, B. J., Goss-Custard, J. D., Hulscher, J. B. and Durell, S. E. A. le V. dit (1996). Causes of variation in prey profitability and its consequences for the intake rate of the oystercatcher *Haematopus ostralegus*. *Ardea* **84A**, 229-268.