RESEARCH ARTICLE

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

Hong-Yan Yang1,2,3,4, Bing Chen5, Zhi-jun Ma6, Ning Hua6, Jan A. van Gils4, Zheng-Wang Zhang1 and Theunis Piersma3,4,*

1Key Laboratory for Biodiversity Science and Ecological Engineering, Beijing Normal University, Beijing 100875, China, 2College of Nature Conservation, Beijing Forestry University, Beijing 100083, China, 3Chair in Global Flyway Ecology, Animal Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, PO Box 11103, 9700 CC Groningen, The Netherlands, 4Department of Marine Ecology, Royal Netherlands Institute for Sea Research (NIOZ), PO Box 59, 1790 AB Den Burg, Texel, The Netherlands, 5Room 2511, Building 1, 2 Nan-Fang-Zhuang, Fengtai District, Beijing 100079, China and 6Coastal Ecosystems Research Station of the Yangtze River Estuary, Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, Institute of Biodiversity Science, Fudan University, Shanghai 200433, China

*Author for correspondence (theunis.piersma@nioz.nl)

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, DMshell). 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 DMshell s⁻¹, i.e. three times higher 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; Verkleu 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 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 fuelling 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 (Luanwan 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 m². 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 m²) 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>
<thead>
<tr>
<th>Table 1. Diet reconstruction</th>
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<tbody>
<tr>
<td>Bivalve species</td>
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<tr>
<td>-----------------------------</td>
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<tr>
<td>Potamocorbula laevis (left shell)*</td>
</tr>
<tr>
<td>Moerella indescens (both shells)</td>
</tr>
<tr>
<td>Macrta quadrangularis (both shells)</td>
</tr>
<tr>
<td>Theora fragilis (both shells)</td>
</tr>
<tr>
<td>Cyclina sinensis (both shells)</td>
</tr>
<tr>
<td>Musculus senhousei</td>
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<tr>
<td>Stenothyra glabra</td>
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<tr>
<td>Nassarius variiferus</td>
</tr>
</tbody>
</table>

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 (DMshell for bivalves and DMtotal for gastropods, g) and ash-free dry mass (AFDMshell for bivalves and AFDMtotal 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$^{-1}$ DMshell) was calculated as: $Q = d \times a \times AFDM_{shell} / DM_{shell}$, 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, $Q = 10^{0.226 \times L - 0.22}$ (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: $E = (r_i-p_i)/(r_i+p_i)$, 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 in 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 (DMshell, g) and ash-free dry mass (AFDMflesh, 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 (DMshell,ingested) to dry mass of droppings retained on a 0.3 mm mesh (DMdroppings):

\[ r = \frac{DM_{\text{shell,ingested}}}{DM_{\text{droppings}}} \]  

(1)

DMshell,ingested was calculated from the proportion of dry shell mass of each prey sample (DMshell,sample) to the flesh mass of the same sample (FMfood,sample) and flesh mass of total food ingested in the trial (FMfood):

\[ DM_{\text{shell,ingested}} = \frac{DM_{\text{shell,sample}}}{FM_{\text{food,sample}}} \times FM_{\text{food}} \]  

(2)

DMshell,sample was calculated as:

\[ DM_{\text{shell,sample}} = \sum_{i=1}^{n} \left( \frac{DM_{\text{shell, size i,subsample}}}{N_{\text{size i,subsample}}} \right) \times N_{\text{size i,sample}} \]  

(3)

where DMshell, size i,subsample and Nsize 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 Nsize i,sample is the number of P. laevis of size class i in each prey sample. The flesh mass of food ingested, FMfood, was calculated as:

\[ FM_{\text{food}} = \frac{FM_{\text{given food}} \times N_{\text{sample}}}{FM_{\text{sample}}} - N_{\text{retained food}} \times FM_{\text{sample}} \times N_{\text{sample}} \]  

(4)

where FMgiven food is the flesh mass of the food given to each experimental bird in a trial, Nsample is the number of P. laevis in each prey sample, and Nretained food is the number of retained food items in the same trial. We used r to correct dry mass of ingested shell (DMshell,ingested) from dry mass of droppings retained on the 0.3 mm mesh (DMdroppings) for the dropping samples collected in the field.

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

\[ SPR = \frac{DM_{\text{shell,ingested}} \times \text{dropping interval}}{N_{\text{droppings}}} \]  

(5)

where Ndroppings 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 droppings 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 covariance 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 Lopires lucinalis at Bane 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 Bane d’Arguin, Mauritania), shell strength was determined using a device built and described by Buschbaum and colleagues (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 0.1N. 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

<table>
<thead>
<tr>
<th>Species name</th>
<th>Family</th>
<th>Average density (individuals m⁻²)</th>
<th>Biomass (g AFDM m⁻²)</th>
<th>% Biomass</th>
<th>Biomass (mg AFDM dropping⁻¹)</th>
<th>% Biomass</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potamocorbula laevis</td>
<td>Corbulidae</td>
<td>15,896</td>
<td>3.13±0.23</td>
<td>69.18</td>
<td>19.02±6.82</td>
<td>92.74</td>
<td>0.01</td>
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<tr>
<td>Moerella indicens</td>
<td>Tellinidae</td>
<td>34</td>
<td>0.18±0.29</td>
<td>3.98</td>
<td>0.92±1.03</td>
<td>4.49</td>
<td>0.08</td>
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<tr>
<td>Mactra quadrangularis</td>
<td>Mactridae</td>
<td>12</td>
<td>0.12±0.28</td>
<td>2.65</td>
<td>0.39±0.31</td>
<td>1.90</td>
<td>0.29</td>
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<tr>
<td>Theora fragilis</td>
<td>Semelidae</td>
<td>16</td>
<td>0.003±0.005</td>
<td>0.07</td>
<td>0.12±0.18</td>
<td>0.59</td>
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<tr>
<td>Musculus senhousei</td>
<td>Mytilidae</td>
<td>5</td>
<td>0.001±0.001</td>
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<td>Stenothyra glabra</td>
<td>Stenothyridae</td>
<td>31</td>
<td>0.36±0.68</td>
<td>7.96</td>
<td>0.02±0.04</td>
<td>0.10</td>
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<tr>
<td>Cyclina sinensis</td>
<td>Veneridae</td>
<td>0.8</td>
<td>0.36±1.09</td>
<td>7.96</td>
<td>0.005±0.01</td>
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<td>Nassaarius variciferus</td>
<td>Nassariidae</td>
<td>1.6</td>
<td>0.19±0.58</td>
<td>4.20</td>
<td>0.003±0.004</td>
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<tr>
<td>Natilia janthostomoides</td>
<td>Naticidae</td>
<td>0.8</td>
<td>0.16±0.48</td>
<td>3.54</td>
<td>0.0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mitrella bella</td>
<td>Columbellidae</td>
<td>0.8</td>
<td>0.02±0.05</td>
<td>0.44</td>
<td>0.0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Assiminea latericea</td>
<td>Assimineidae</td>
<td>3</td>
<td>0.0004±0.001</td>
<td>0.01</td>
<td>0.0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* 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 m² (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; χ²=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 DMshell (N=2795); the quality of the most frequently eaten length class (3 mm) was 1.32 kJ g⁻¹ DMshell (Fig. 2). This is only about a third of the predicted value of 3.5–4.5 kJ g⁻¹ DMshell needed by birds to maintain a stable gizzard mass (5.9 g) between departure from NW 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 DMshell (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 2.73 s⁻¹. This is much lower than the estimated requirement of 3.63 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.
The median of the dropping intervals was 79 s\(^{-1}\) (Fig. 5). The slope of the regression (i.e. \(r\)) (Dekinga and Piersma, 1983) was estimated at 2.3. Therefore, the estimate of \(\text{DM}_{\text{shell, ingested}}\) of \(P.\ laevis\) had to be corrected by multiplying \(\text{DM}_{\text{droppings}}\) 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 (\(\chi^2=4.27,\ d.f.=1,\ P=0.043\)). 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 5.11 Js\(^{-1}\), a value that is higher than the predicted minimum energy intake rate of red knots staging in Bohai Bay (3.61 Js\(^{-1}\)). A metabolizable energy intake rate of ca. 5 Js\(^{-1}\) is similar to the values for red knots in the Dutch Wadden Sea: 5.5 Js\(^{-1}\) (on a diet of \(M.\ balthica\)) (Zwarts and Blomert, 1992; Piersma et al., 1994) and higher than 3.6 Js\(^{-1}\) 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 Js\(^{-1}\)) (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
the (ingested size-specific) ratio \( r \) and measured breaking force (Spearman’s rank test, \( p=0.097, \; \hat{r}=0.005 \); 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 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* (2.7±0.5 mg, mean ± s.e.m., \( N=43 \)) 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 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 ± 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 ± s.e.m., \( N=9 \)).
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. 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:

\[
\text{DM}_{\text{shell, ingested}} = r \times \text{DM}_{\text{droppings}}, \quad (A1)
\]

\[
\text{AFDM}_{\text{ingested}} = \text{DM}_{\text{shell, ingested}} \times \alpha, \quad (A2)
\]

where dry mass is in g and \(\alpha\) is the ratio of ash free dry mass of flesh to dry mass of ingested prey. EIR of the two red knot subspecies in Bohai Bay was calculated in a series of steps as follows:

\[
\text{AFDM}_{\text{ingested}} = \text{DM}_{\text{shell, ingested}} \times \alpha, \quad (A2)
\]

where dry mass is in g and \(\alpha\) is the ratio of ash free dry mass of flesh to dry mass of ingested prey. (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 \(R^2=0.59, F_{1,3}=6.72, P=0.08\).

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AUTHOR CONTRIBUTIONS
All authors contributed to the design of the study. H.-Y.Y., B.C., N.H. and T.P. collected data, H.-Y.Y. and T.P. together with J.A.V.G. wrote the manuscript with important input from Z.-J.M. and Z.W.Z.

COMPETING INTERESTS
No competing interests declared.

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REFERENCES


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Fig. S1. Estimating shell length from the height of hinge and top for the main food of red knots in Bohai Bay: *Potamocorbula laevis*. The shapes of the two valves of *P. laevis* are different, and as a result the relationships between height of hinge and top and shell length differ within one bivalve. We thus only measured left shells.