INTRODUCTION

Organisms respond adaptively to short-term changes in their environment by behavioural, physiological, and morphological adjustments (Piersma & Lindström 1997, Piersma & Drent 2003). For example, in order to increase digestive efficiency, tadpoles grow longer guts when competition with other tadpoles strengthens (Relyea & Auld 2004). If such intra-individual changes are reversible, we generally speak of ‘phenotypic flexibility’ (Piersma & Drent 2003). Recently, there has been increasing attention paid to phenotypic flexibility, notably for reversible size changes of nutriti...
tional organs (Overgaard et al. 2002, Bozinovic et al. 2003), and especially so among ornithologists (Starck 1999, McWilliams & Karasov 2001, Guglielmo & Williams 2003, López-Calleja & Bozinovic 2003, Tieleman et al. 2003, McWilliams & Karasov 2005). For example, long-distance avian migrants are able to breakdown their digestive system before take-off in the course of just a week, while they rapidly build-up these organs again when fuelling along the route (Piersma 1998, Piersma & Gill 1998).

Now that documentation of flexibility in the digestive system is accumulating for a wide range of taxa, the time seems ripe to study this phenomenon from an optimality point of view. Thinking of phenotypic flexibility as ‘physiological decision-making’ should yield insights into the currencies and constraints underlying organ size changes, just as looking at foraging from an optimality perspective has, over the past four decades, taught us a great deal about the currencies and constraints underlying foraging decisions (reviewed by Stephens & Krebs 1986, Perry & Pianka 1997, Vásquez & Kacelnik 1998, Stephens et al. 2007). Specifically, with respect to flexible adjustments of the gastrointestinal tract, the more recently developed optimal digestion theory (Penry & Jumars 1987, Jumars 2000, Logan et al. 2002) may become the predictive framework for empirically oriented studies on flexible digestive systems.

Central to each optimality problem are the costs and benefits associated with each possible option. Here, the subject of study itself, reversible phenotypic variation, comes as a powerful tool to be used in experimental studies (Sinervo & Basolo 1996, Piersma & Drent 2003). By manipulating and (ideally) tracking size changes in digestive organs within individuals, (energetic) costs and benefits can elegantly be expressed as a function of variation in organ size within individuals (comparable to manipulative foraging studies mentioned above).

Red Knots *Calidris canutus*, medium-sized shellfish-eating shorebirds, are an ideal species upon which to undertake such optimality analyses of digestive organs. During the non-breeding phase of life (10 months a year), they dwell on intertidal mudflats, feeding on hard-shelled molluscs, which they ingest whole (Piersma et al. 1993, Fig. 1). Because of this particular feeding habit, they possess a large muscular gizzard, which they use to crush their heavily armoured prey (Battley & Piersma 2005). Opposing evolutionary forces, favouring temporary reductions in gizzard and gut size (Piersma et al. 1999), are their long-distance migrations, which they undertake twice a year between breeding and non-breeding grounds (5000–15 000 km, one-way). Knots can be kept in captivity quite easily, and in recent years, experiments with captive birds have shown the ability to measure gizzard sizes in live birds using ultrasonography (Dietz et al. 1999), to manipulate gizzard size by the food on offer (Dekinga et al. 2001), and to measure the costs (Piersma et al. 2003, 2004) and benefits (van Gils et al. 2003a, 2003b, 2004, 2005a, 2005b, 2005c, van Gils & Piersma 2004) of feeding and digestion.

In this paper, we will review the optimality analyses that we performed on the gizzards of Knots. We do so by presenting the crucial experiment that mechanistically revealed the dependence of digestive processing rate on gizzard mass (experiment 1 in van Gils et al. 2003a). Subsequently, we formalise the observed relationships between gizzard masses and their associated energetic costs and benefits. We suggest two mutually exclusive currencies that may underlie the observed seasonal shifts in gizzard mass. Finally, the resulting optimality model is tested using field data on gizzard masses of Red Knots collected over different temporal and spatial scales (between years, within years, and between sites).

**METHODS**

**An experiment on digestive processing rates**

Six captive Red Knots, caught in the Dutch Wadden Sea in 1994–99, were randomly assigned to two flocks of each three individuals (experiment performed in August 2000). In order to manipulate gizzard size we followed the procedures out-
Dekinga et al. (2001), who found that, in the course of a single week, Knots enlarge their gizzards when offered hard-shelled prey, while Knots reduce their gizzards when offered soft food. Based on these results, the flock that was intended to develop large gizzards was given hard-shelled Edible Cockles *Cerastoderma edule*, while the flock that was supposed to maintain small gizzards was given soft trout pellets (Trouvit, Produits Trouw, Vervins, France). These specific staple foods were offered to the birds three weeks before the actual experiment started.

At three times we used an ultrasound to estimate gizzard mass (Pie 200 ultrasound, Pie Medical Benelux BV, Maastricht, The Netherlands; see Dietz et al. 1999 for detailed methodology): first, before gizzard manipulation started, in order to confirm similarity in gizzard mass between the two groups \( (P > 0.3) \); second, at the onset of the experiment, and third just after the experiment had ended, both in order to confirm the success of gizzard-size manipulation \( (P < 0.01; \text{HLM with 6 level–2 observations}) \).

The experiment consisted of 36 trials \((6 \text{ birds } \times 6 \text{ trials/bird})\), where in each trial a single Knot was offered a single prey type *ad libitum* (such that intake rate would not be constrained by searching). In total, we offered 6 prey types, hence 6 trials per bird \((3 \text{ size classes of Edible Cockles and Baltic Tellins } \textit{Macoma balthica}; \text{ size classes were 5–7 mm, 9–11 mm, and 13–15 mm of shell length})\). Each trial lasted about 40 minutes, which

Figure 1. Red Knots ingest their mollusc prey whole and therefore need a relatively large gizzard in order to crush this hard-shelled food (photo by Jan van de Kam).
was enough to consume on average about 40 prey items. As shell crushing usually commences after 3–9 prey ingestions, the duration of the trials was sufficient for intake rates to be potentially constrained by rates of shell crushing. Trials were performed in random order with respect to bird and prey type.

Each trial was videotaped, using a Hi–8 video camera. We used ‘The Observer’ package (Noldus Information Technology 1997) to analyse prey intake in full detail. Replaying the tapes at 1/5 of the recording speed, we scored each prey intake and the time it took to handle each prey item (to the nearest 0.04 s). Handling times were measured in order to test for the hypothesis that intake rates were constrained by rates of handling rather than by rates of digestive processing (see van Gils et al. 2003a).

We took samples from the six prey types that we offered, in order to measure dry shell mass (DMshell). We did so by taking out all fleshy parts of individual prey items before drying the shells for three days in a ventilated oven at 55–60°C, where after DMshell was determined to the nearest 0.1 mg.

Each trial yielded one datum on intake rate (prey s−1). Data were normalised by log-transformation and were analysed using the GLM-procedure in SYSTAT 10 (Systat Software Inc.). As in some trials birds ingested no prey at all, we added 0.001 prey s−1 to each intake rate in order to enable log-transformation of 0 values (following Berry 1987). A series of GLM-models were run, testing whether handling time or DMshell constrained rate of prey intake. Here we will only present the most parsimonious model that remained, in which intake rate varied only with gizzard mass and DMshell but not with handling time. For more details about the statistical analyses and further methodology we refer to van Gils et al. (2003a; experiment 1).

An optimality model on gizzard size

Using the outcome of the experiment described above enabled us to model maximal digestive processing rate (g DMshell s−1) as a function of gizzard mass. Subsequently, knowing a prey type’s metabolisable energy content per g DMshell allowed us to model metabolisable energy intake rate as a function of gizzard mass under a digestive bottleneck (for a fictive example of such a function see Fig. 2). These energetic benefits B as a function f of gizzard mass G can formally be equated as:

\[ B = f(G) \]  

Function f will be parameterised in the current paper.

Along the same lines, we can formalise metabolic costs C as a function h of gizzard mass G (Fig. 2):

\[ C = h(G) \]

For the parameterisation of function h we refer to the appendix in van Gils et al. (2003a). Basically, C increases with an increase in G because maintenance and transport costs and heat increments of feeding are larger in larger gizzards (see also Piersma et al. 2003).

Next, we define two reasonable currencies and optimise G with respect to B and C (Fig. 2). Firstly, we imagine so-called satisficing foragers, which aim to maintain energy balance on a daily basis.
(i.e. keep body mass stable), hence:

\[ B - C = 0 \]  

(3)

Secondly, we envisage so-called net-rate maximising foragers, which aim to maintain a daily energy budget as positive as possible (i.e. fuel at maximum rate), hence:

\[ \max(B - C) \]  

(4)

We expect Red Knots to behave as satisficers during times when body mass is relatively stable (at the wintering grounds, long before the onset migration), while we expect Knots to behave as rate-maximisers when fuelling for migration (either at wintering grounds or at stopovers).

Field data on gizzard masses

The optimality model was tested using field data on gizzard masses collected over the years. Basically, we gathered three types of data on gizzard masses in Red Knots. (1) Data collected for several years (1998–2002) in late summer (late July–August) in the Dutch Wadden Sea \( (n = 564) \), which were all obtained through ultrasonography on live birds. (2) Data collected during different times of the annual cycle in the Wadden Sea \( (1984–2002; n = 920) \), of which 73 were obtained through dissection of carcasses and the rest through ultrasonography on live birds. (3) Data collected worldwide at several wintering grounds and stopover sites on five out of the six recognised subspecies of Knots \( (n = 7) \), namely:

- *canutus* at Mauritanian wintering ground, \( n = 6 \);
- *canutus* at Wadden Sea stopover, \( n = 2 \);
- *islandica* at Mauritanian wintering ground, \( n = 6 \);
- *islandica* at Wadden Sea stopover, \( n = 2 \);
- *islandica* at Wadden Sea wintering ground, \( n = 60 \);
- *islandica* at Icelandic stopover, \( n = 8 \);
- *piersmai* at NW-Australian wintering ground, \( n = 24 \);
- *rogersi* at New Zealand wintering ground, \( n = 5 \);
- *rufa* at Tierra del Fuego wintering ground (S-Argentina), \( n = 13 \);
- *rufa* at San Antonio Oeste stopover (E-Argentina), \( n = 7 \);

ultrasonography was used on all wintering *islandica* and 20 *piersmai* individuals, all other gizzard masses were obtained through carcass analysis. Carcasses were either collected as catching casualties, or recovered from poachers \( (rogersi) \), or obtaining by shooting under license \( (islandica \text{ in Iceland}) \).

RESULTS

An experiment on digestive processing rates

Intake rates \( I \) (prey s\(^{-1}\)) increased with gizzard mass \( G \) (g) and decreased with the amount of shell mass per prey \( DM_{\text{shell}} \) (mg) \( (n = 36, R^2 = 0.82, P < 0.00001) \). In the most parsimonious GLM-model, the coefficients of log-transformed \( G \) and \( DM_{\text{shell}} \) did not deviate from 2 and –1 respectively \( (P > 0.9 \text{ and } P > 0.1; \text{ see van Gils et al. 2003a for the results of more complicated GLM-models}) \). Therefore, once backtransformed, the observed relationship can be formalised as:

\[ I = c \frac{G^2}{DM_{\text{shell}}} \]  

(5)

where \( c \) is constant at 0.05 mg \( DM_{\text{shell}} \text{ s}^{-1} \text{ g}^{-2} \text{ gizzard} \). The inverse relation between \( I \) and \( DM_{\text{shell}} \) suggests that the rate at which shell mass is digestively processed \( (mg DM_{\text{shell}} \text{ s}^{-1}) \) is constant for a given gizzard mass. Therefore, if we define shell mass processing rate as:

\[ P = I \times DM_{\text{shell}} \]  

(6)

we can simplify equation (5) to:

\[ P = c \times G^2 \]  

(7)

For clarity we have calculated shell mass processing rate \( P \) in each trial and plotted its dependence on gizzard mass \( G \) (Fig. 3).

Fine-tuning the gizzard model with empirical insights

Now we know that digestive processing rate relates in a rather simple manner to gizzard mass and a characteristic of the prey \( (\text{shell mass } DM_{\text{shell}}) \), constructing the optimisation model of gizzard size is, at least from the benefit side of the coin, a rather straightforward exercise. If we define \( m \) as a second characteristic of the prey, namely the amount of metabolisable energy per g shell mass \( (\text{from now on this ratio will be called prey quality}) \), equation (7), describing digestive processing rate \( P \) in terms of shell mass per unit time, can be expressed as \( E \) in terms of metabolisable energy uptake per unit time:
\[ E = m \times P \]

hence (combining equations 7 and 8):

\[ E = m \times c \times G^2 \]  

This latter equation forms the heart of the optimisation model, as it couples the energetic benefits to the cost-component of the calculations. Namely, under the assumption of *satisficing*, \( E \) should equal metabolic rate in order to keep body mass constant (when calculated on a daily basis). Thus, if we define \( E_{\text{required}} \) as metabolisable energy intake rate *required* to maintain energy balance, we can, by rewriting equation (9), calculate the gizzard mass \( G_{\text{required}} \) *required* to achieve this:

\[ G_{\text{required}} = \sqrt{\frac{E_{\text{required}}}{mc}} \]  

A graphical example on how \( E_{\text{required}} \) and \( m \) lead to a predicted \( G_{\text{required}} \) for *satisficers* is given in Fig. 4 (filled dot).
Comparing model predictions with field data
Based on the literature and occasionally on unpublished data, we estimated \( m \) and \( E_{\text{required}} \) for each specific site and time of year where we had data on gizzard masses available (see van Gils et al. 2003a, 2005a, 2006a for references). Estimates of \( m \) were made by combining prey type-specific values for \( m \) with estimates of diet composition as determined by dropping analysis (Dekinga & Piersma 1993). \( E_{\text{required}} \) basically varied with expected thermoregulatory costs (using Wiersma & Piersma 1994) and with time available for foraging (12–16 h depending on time of year and site). We assumed satisficing during overwintering and rate-maximising when stopping over and when fuelling to depart from the wintering grounds.

In all three comparisons (between years, within years, between sites), the fit between observations (y) and predictions (x) was strong (Fig. 5; between years: \( n = 564, R^2 = 0.15, P < 0.00001, y = 0.00 + 1.00x \); within years: \( n = 920, R^2 = 0.23, P < 0.00001, y = 0.44 + 0.94x \); between sites: \( n = 125, R^2 = 0.35, P < 0.00001, y = 1.42 + 0.83x \)). As predicted, gizzards were generally small (5–7 g) in islandica wintering in the Wadden Sea in late summer (especially in the first years of the dataset), and in piersmai wintering in NW-Australia (Roebuck Bay). Gizzards were large (10–11 g) in islandica fuelling in the Wadden Sea in early spring, and in canutus wintering in Mauritania (Banc d’Arguin).

Two issues are worth pointing out here. Firstly, largest deviations from expectations (underestimations by 1.6–2.0 g) were found in islandica in the Wadden Sea in early winter (Oct–Dec), which is when we assumed satisficing as a currency. Secondly, to our initial surprise, rate-maximising gizzards are not larger than satisficing gizzards in the ‘between-sites’ comparison (\( R^2 = 0.45, P > 0.1 \), taking subspecies into account).

**DISCUSSION**
This study shows that modelling costs and benefits that come with the size of a certain organ can be

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**Figure 5.** Relation between observed gizzard mass and predicted gizzard mass at various temporal and spatial scales: (A) between years (Dutch Wadden Sea during late summer); (B) between months (Dutch Wadden Sea; number indicates month) and (C) between sites (worldwide; letter indicates subspecies: c = canutus, i = islandica, p = piersmai, ro = rogersi, and ru = rufa). In all plots, black dots indicate samples from wintering periods when satisficing gizzards are predicted; in (B) and (C) open dots indicate fuelling periods when rate-maximising gizzards are predicted; additionally, in (B), grey-filled dots indicate those months when Knots build up a small peak in body mass (see Discussion). Diagonal lines give \( y = x \) lines and bars give standard errors.
effective. We took advantage of phenotypic flexibility by experimentally manipulating gizzard size in order to measure its performance over a size-range. This performance function formed the basis of our optimality model, which successfully predicted gizzard masses of Red Knots at various temporal and spatial scales. Just as tests of optimality models in behavioural ecology yield insights into the currencies and constraints underlying a behavioural decision (Stearns & Schmid-Hempel 1987, Mitchell & Valone 1990; see van Gils et al. 2006b for an application), our tests generate evidence for the currencies and constraints underlying the physiological decision to adjust gizzard size.

With respect to currencies, we found that Knots behave as so-called satisficers throughout most of the year and only behave as rate-maximisers during fuelling. In the light of their seasonal changes in body mass this truly makes sense, but it may come as a surprise to foraging theoreticians who assume rate-maximisation, and not satisficing, to be the rule rather than the exception (e.g. McNamara et al. 1993, but see Ward 1992). In this perspective, it is worth pointing out that islandica Knots during early winter follow a sort of intermediate currency: their gizzards are larger than required for balancing the energy budget (0.8–2.0 g larger; grey dots in Fig. 5B), while they are smaller than required for maximising the energy budget (on average, respectively 1.4 g smaller in October, 2.3 g smaller in November, and 1.4 g smaller in December; see van Gils et al. 2003a for more details). In agreement with this is the observation that islandica shows some increases in body mass in early winter (presumably as a way to cope with increased unpredictability in food supply and demand during midwinter; Piersma 1994, see also van Gils et al. 2006).

With respect to constraints, this study comes with overriding evidence that intake rates in Red Knots are constrained by rates of digestion. Our calculations suggest that, throughout their annual cycle, Knots continuously adjust the capacity of their digestive machinery such that it is just sufficient to process the daily required amount of energy (be it in order to balance or in order to maximise the energy budget). Again, this sheds new light on many classic and current perspectives on foraging, which have, by taking Holling’s disc equation as a basis (Holling 1959), assumed (interference-free) intake rates to be constrained by rates of encounter and handling (Piersma et al. 1995, Norris & Johnstone 1998, Caldow & Furness 2001, Gill et al. 2001, van Gils et al. 2004; but see recent reviews by Jeschke et al. 2002 and Karasov & McWilliams 2005). Not only does this ‘Holling-point-of-view’, at least in the case of Red Knots, lead to biased predictions on intake rates (van Gils et al. 2003a), it also leads to a misunderstanding of the mechanism driving prey selection (van Gils et al. 2005b). In a multiple prey environment, prey selection according to Holling’s model is based on prey profitability, i.e. only feed on those prey types whose energy content per unit handling time exceeds long-term energy intake rate (Pulliam 1974, Charnov 1976). However, when digestively constrained, prey should not be picked out on the basis of profitability, but rather on the basis of prey quality, i.e. the amount of energy content per g indigestible matter (Hirakawa 1995).

Along an expanding spatial scale, prey selection drives patch selection and possibly even (stopover-) site selection and therefore a proper mechanistic understanding of prey selection is of critical importance in distributional ecology. Indeed, differential patch selection by individual Red Knots in the Wadden Sea could be explained by the fact that digestively constrained individuals selected patches containing prey of high quality, while digestively unconstrained individuals selected patches on the basis of prey profitability (van Gils et al. 2005c). And indeed, at the largest spatial scale, migratory Knots even seem to pick out their stopover sites on the basis of prey quality (van Gils et al. 2005a). By stopping over at such ‘hotspot’ stopover-sites, fuelling at maximal rate is feasible with relatively small gizzards (all around 8 g; Fig. 5C). This saves them costly time, involved in adjusting gizzard size (Dekinga et al. 2001). As Knots fly with ultra-light gizzards (usually around 6 g; in order to minimise maintenance and transport costs), the selection of a hotspot will enable
them fuel at full speed after relatively little 'gizzard-adjustment-time'. And, upon departure from the hotspot, they would lose little time shifting back to a 'cheap-flight gizzard'.

So, besides maximum rate of digestion itself, the time it takes to enlarge digestive capacity such that maximum rates of digestion can be achieved seems to be an important additional constraint (Karasov & McWilliams 2005, McWilliams & Karasov 2005). With respect to post-breeding _islandica_ arriving in the Wadden Sea in late summer, we found that survival chances critically relate to this 'gizzard-adjustment time'. Upon arrival, gizzards are often undersized (i.e. $G < G_{\text{satisficing}}$). The energy budget of Knots with undersized gizzards is negative and therefore the time available for adjusting gizzard mass depends on the amount of energy stores left. From field data on 'gizzard growth rates' (Piersma _et al._ 1999), we calculated that Knots arriving with undersized gizzards have fat stores left that allow them to regrow their gizzards by one gram only. Using data on gizzard masses upon arrival, we could therefore predict the proportion of birds that would be able to survive on a given prey quality: only those birds arriving with a gizzard mass $G \geq G_{\text{satisficing}} - 1$. As an increase in prey quality leads to a decrease in $G_{\text{satisficing}}$ (Fig. 4), we expected the proportion of birds surviving to increase with prey quality. Indeed, calculated from resighting rates of colour banded Knots (using MARK-software), annual survival rates (range: 0.45–0.72 year$^{-1}$) matched our quantitative predictions and increased with inter-annual variation in prey quality (range: 1.78–2.61 J mg$^{-1}$ DM$_{\text{shell}}$) in a way which suggests that, upon arrival, there is room for flexibly increasing gizzard size by one gram only (van Gils _et al._ 2006a).

To conclude, the study presented here shows that modelling the physiological decision underlying organ-size shifts can be effective and insightful. It leaves many open questions, which are relevant to address in the future. Firstly, given the importance of the time course of reversible organ-size changes, we need to know the factors determining rates of change in gizzard mass. Why did gizzards under well-nourished laboratory conditions (Dekinga _et al._ 2001) grow by more than an order of magnitude faster than those observed in the field (Piersma _et al._ 1999)? Secondly, given that survival chances seem to depend on gizzard mass upon arrival, we need to know what sets arrival gizzard mass. Length of migration and availability of potential stopover sites along the route play critical roles (Piersma 1998, Piersma _et al._ 2005), but also stochasticity in environmental conditions should matter. For example, what is the effect of expected prey quality at the next stopover? Here, modelling techniques such as stochastic dynamic programming (Houston & McNamara 1999, Clark & Mangel 2000) can help us elucidating how digestive organ size during long-distance flights should be optimised with respect to such unpredictability _en route_.

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SAMENVATTING

Er bestaat een toenemende belangstelling onder ornithologen voor omkeerbare fenotypische veranderingen, in het bijzonder voor grootteveranderingen in het maagdarmkanaal van langeafstandstrekkers. In dit artikel presenteren we een overzicht van de kosten-baten-analyses die we maakten van de flexibele spiermaag van de Kanoet *Calidris canutus*. Door de hardheid van het aangeboden voedsel te variëren, manipuleerden we de grootte van de spiermaag. Dit stelde ons in staat de energetische kosten en baten van foerageren uit te drukken als functie van de spiermaaggrootte. Deze functies maakten het mogelijk de optimale spiermaaggrootte te modelleren, zowel voor Kanoeten op trek als tijdens de winter. We namen hierbij aan dat Kanoeten of (i) probeerden hun dagelijkse energiebudget in *balans* te houden, of (ii) probeerden hun dagelijkse energiebudget te *maximaliseren*. Het model voorspelde nauwkeurig de variatie in spiermaaggrootte die we vonden (1) tussen jaren, (2) binnen jaren en (3) tussen plekken. Het bleek dat de magen van Kanoeten in de winter geschikt waren om het energiebudget in *balans* te houden, terwijl ze in de opvetperiodes voor de wegtrek groot genoeg waren om het budget te *maximaliseren*. Deze toetsing van het magenmodel geeft het belang aan van verteringsbeperkingen en prooikwaliteit in het leven van Kanoeten.

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