# TIME COURSE AND REVERSIBILITY OF CHANGES IN THE GIZZARDS OF RED KNOTS ALTERNATELY EATING HARD AND SOFT FOOD

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## Summary

The ability to change organ size reversibly can be advantageous to birds that perform long migrations. During winter, red knots (*Calidris canutus*) feed on shellfish and carry a muscular gizzard that weighs 10 % of their body mass. Gizzard size decreases when these birds eat soft foods, e.g. while breeding in the tundra. We studied the reversibility and time course of such changes using ultrasonography. Two groups of shellfish-adapted knots (*N*=9 and *N*=10) were fed alternately a hard and a soft food type. Diet switches elicited rapid reversible changes. Switches from hard to soft food induced decreases to 60 % of initial gizzard mass within 8.5 days, while switches to

hard food induced increases in gizzard mass to 147 % within 6.2 days. A third group of knots (*N*=11), adapted to soft food for more than 1 year, initially had very small gizzards (25 % of the mass of shellfish-adapted gizzards), but showed a similar capacity to increase gizzard size when fed shellfish. This is the first non-invasive study showing rapid digestive organ adjustments in non-domesticated birds.

Key words: gizzard, mass fluctuation, red knot, *Calidris canutus*, diet, ultrasonography.

## Introduction

Even in homeothermic vertebrates with determinate growth, organ size is far from constant. Organs can show reversible transformations in response to changes in the environment or in the behaviour of the animal. Only recently has such 'phenotypic flexibility' (intra-individual adjustment of organ size to variable ecological contexts) been brought within the realm of evolutionary biology (Ricklefs, 1991; Karasov, 1996a; Karasov, 1996b; Secor and Diamond, 1998; Secor and Diamond, 2000). The existence of phenotypic flexibility makes it possible to study the processes that match organ size and performance at the individual level (Piersma and Lindström, 1997; Starck, 1999a; Starck, 1999b).

Animals living in seasonally changing environments and birds that migrate may experience conditions that favour the existence of organ flexibility (Spitzer, 1972; Biebach, 1998; Piersma, 1998). For instance, red knots *Calidris canutus* carry a gizzard that constitutes 10 % of their body mass (Piersma et al., 1993b). There must be good reasons to carry and pay the maintenance costs of such a heavy organ. Red knots use the gizzard to crush hard-bodied prey, and thus the reason seems to be that red knots feed mainly on molluscs (Piersma et al., 1993a; Piersma et al., 1999a). However, during the short summer season on the tundra, they eat soft-bodied surface arthropods and spiders (Tulp et al., 1998). Another molluscivore shorebird, the great knot *Calidris tenuirostris*, has been shown to carry a smaller gizzard in

summer than in winter (P. S. Tomkovich in Piersma et al., 1999a). During migration, the gizzard of the red knot seems to be able to change size rapidly (Piersma et al., 1993b; Piersma et al., 1999b).

The seasonal changes in gizzard size in red knots have been documented by dissection of collected specimens, but dissection cannot be used to examine the time course and reversibility of internal organ size changes without killing a large number of birds. Starck (Starck, 1999a) used ultrasonography, an non-invasive method, and dissection to record reversible changes in gizzard size of domesticated Japanese quail *Coturnix japonica*. In this study, we used ultrasonography (Dietz et al., 1999a) to investigate the time course of gizzard size changes in red knots alternately fed a hard and a soft food type. We aimed to determine (i) whether, within individuals, a diet of hard-shelled molluscs leads to a large gizzard whereas a diet of soft food pellets leads to a small gizzard, (ii) whether these changes are reversible and (iii) how fast these changes occur.

## Materials and methods

### Birds and housing

Nineteen red knots *Calidris canutus islandica* were caught with mist-nets on 19 and 20 February 1999 on intertidal flats

Table 1. *Body mass, body size variables and gizzard mass of the three groups of knots at the start of the experiment*

| Group | Body mass (g) | Wing length (mm) | Bill length (mm) | Tarsus length (mm) | Gizzard mass (g) |
|---|---|---|---|---|---|
| A | 126.3±2.4 | 169±2 | 32.5±0.5 | 31.0±0.2 | 7.99±0.26 |
| B | 125.4±2.7 | 173±3 | 32.8±0.7 | 31.1±0.5 | 8.03±0.33 |
| C | 125.5±3.5 | 170±2 | 33.3±0.8 | 31.4±0.4 | 2.57±0.28 |

Values are means ± S.E.M.; $N$=10 for group A, $N$=9 for group B and $N$=11 for group C.

in the western Dutch Wadden Sea. Body size and mass were measured. The birds were transported to the shorebird facilities at the Netherlands Institute for Sea Research (NIOZ), Texel, The Netherlands. We immediately determined gizzard size by ultrasonography.

The sample size necessary to detect differences among groups in mean gizzard size at $P$=0.05 was determined following Sokal and Rohlf (Sokal and Rohlf, 1997) on the basis of past experience (Dietz et al., 1999a). The birds were allocated to two groups (group A, $N$=10; group B, $N$=9) that were made comparable with regard to body size, initial body mass and gizzard mass (Table 1; $N$=19, consisting of all birds in groups A and B, ANOVA, $F_{1,17}<1.3$ and $P>0.1$ for all). A third group of 11 red knots (group C) consisted of individuals captured in the western Wadden Sea area in 1994, in 1995 or in 1998 and maintained in captivity on a food pellet diet. The initial body mass and size of these latter birds was similar to that of the freshly caught birds at the onset of the experiment (Table 1; $N$=30, ANOVA, $F_{2,27}<1.1$ and $P>0.1$ for all).

During the experiment, the three groups were housed in identical but separate outdoor aviaries (4 m×2.5 m×2.5 m). Birds had access to a small barren artificial mudflat in which they probed frequently without food reward. A bath of fresh water was available. After the experiment, the birds of groups A and B were released back into the wild.

*Diet*

In the Wadden Sea, red knots forage mainly on shellfish, a hard food type (Zwarts and Blomert, 1992; Piersma et al., 1993a). We therefore assumed that, prior to the experiment, the diet of the recently caught birds (groups A and B) also consisted of shellfish. In captivity, these birds were fed one of the two experimental diets (see below). The long-term captive birds (group C) had been eating trout pellets (Trouvit, Produits Trouw, Vervins, France), a soft food, for at least 1 year.

The two experimental diets consisted of (i) trout pellets, to mimic a relatively soft food source such as tundra arthropods and young shorecrabs *Carcinus maenas*, or (ii) blue mussels *Mytilus edulis*, which represent a hard-shelled food type. Small mussels were collected at the wave breakers on the North Sea beach of Texel. Before being given to the birds, the very dense mussel clusters were broken up and the loose mussels cleaned. The birds were able to select from the original length distribution with a maximum of 25 mm (which red knots are still able to swallow; Zwarts and Blomert, 1992). The size and quality of the available mussels varied from day to day. Diet

switches were always complete; we never offered the two different food types on the same day.

*Experimental protocol*

The diet of group A was switched twice and that of group B four times (Fig. 1). The experiment started when one group (group B in Fig. 1) was switched from hard (mussels) to soft food (trout pellets). This group was kept on pellets until gizzard
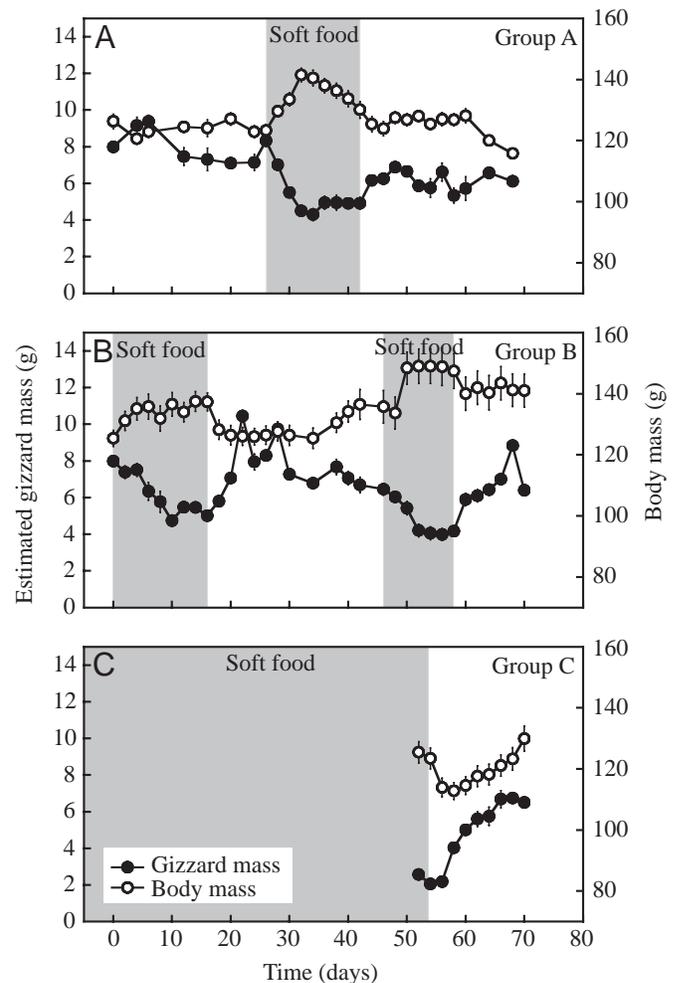


Fig. 1. Variation in gizzard mass (●) and body mass (○) for (A) group A ($N$=10), (B) group B ($N$=9) and (C) group C ($N$=11). Values are means ± S.E.M. The experimental diet is indicated in each panel by non-shaded (hard mussel diet) and shaded (soft trout pellet diet) areas.

size had reached a plateau over a minimum of three consecutive measurements (i.e. 4 days). The group was then switched back to mussels. When the average gizzard size of group B had reached a new plateau, food type was switched in group A, which had so far been used as a reference group. When the gizzard size of group A had reached a stable low level, these birds were switched back to mussels. When group A reached a stable enlarged gizzard size, group B was again subjected to the whole sequence of switches from hard mussels to soft pellets and back, as described above. In the absence of appropriate statistical tools to deal with the dependence of the results of the first and second series of switches of group B, these two trials were treated as independent.

To verify whether birds that had not been exposed to hard-shelled food for 1 year or more would still be able to adjust gizzard size to accommodate a switch to mussels, group C was used. At day 54 of the entire experiment, these birds were given their first mussels. Group C was kept on the mussel diet until average gizzard size had clearly reached a plateau.

### Ultrasonography

Gizzard size was determined by ultrasonography every second day in the trial group(s) and every fourth day in the reference group. Prior to the measurements, food was withheld from the birds for 2 h to ensure that their gizzards were empty (Weber and Piersma, 1996). Birds were selected at random from the groups, and body mass was determined on an electronic balance to the nearest 1 g. As in a previous study on changes in pectoral muscle mass (Lindström et al., 2000), measurements were performed blindly, with the observer (A.D.) being unaware of the identity and the treatment group of the birds. Furthermore, the observer was not able to see the values of the actual size measurement on the screen.

Measurements were taken using a Pie 200 ultrasound apparatus with a 7.5 MHz linear probe (Pie Medical Benelux BV, Maastricht, The Netherlands) and an ultrasonic gel to couple the probe to the surface of the animal. The images were printed on a Mitsubishi video copy processor (model P90E). The width and height of the gizzard (±0.1 mm accuracy according to the manufacturer) were measured by placing the probe transversely on the belly of the bird, at an angle of approximately 45 ° just below the rib case (see figs 1C, 2C,D in Dietz et al., 1999a). To increase the reliability of the measurements, gizzard size was measured twice per bird by placing the probe anew on the bird (Dietz et al., 1999a; Starck et al., 2001). The ultrasonographic scans took approximately 5 min per bird, after which the contact gel was removed from the feathers with lukewarm water. During the whole experimental period of 70 days, no skin or feather problems were observed.

Prior to the experiment, calibration curves were made for the particular observer using 21 dead red knots with widely variable gizzard sizes (see Dietz et al., 1999a). Gizzard width was used as an estimate of gizzard mass, because gizzard width appeared to be the most reliable predictor of gizzard mass for the present observer. Following Dietz et al. (Dietz et al., 1999a), gizzard width (*W*; cm) measurements were converted to gizzard mass (*M*; g) as: $M = -5.35 + 7.88W$ ($N=21$, $r^2=0.700$, $P<0.001$). Cubical transformations did not improve the fit. The repeatability of the stomach width measurements during calibration was 0.66 (calculated following Lessells and Boag, 1987). This estimate is similar to repeatabilities achieved by another observer (Dietz et al., 1999a).

### Statistical analyses

Results are presented as means ± S.E.M. The means of two groups were compared using analysis of variance (ANOVA) (SPSS 9.0), and the Ryan–Einot–Gabriel–Welsch multiple-range test (REGWQ, univariate analysis of variance procedure, α set at 0.05, SPSS 9.0) was used to compare the means of three or more groups. When the groups differed significantly from each other, the output showed the division of the groups over the subsets found.

We expected that when gizzards reached the size appropriate for a particular food type, up- or downregulation would stop. Thus, plots of gizzard size over time should show clear breakpoints and, in our opinion, they did (see Fig. 2). We therefore applied a continuous biphasic linear regression model to calculate the rates of increase or decrease in gizzard mass after a switch of diet. This model also makes it possible to estimate the time at which a new plateau in gizzard mass has been reached and the value of the plateau. Using the non-linear regression algorithm procedures of NONLIN (shareware program, P. H. Sherrod), the parameters of the model (see Koops and Grossman, 1993; Dietz and Drent, 1997) were calculated as follows:

$$M = \alpha + \beta_1 t - [r(\beta_1 - \beta_2)\ln(1 + e^{(t-\gamma)/r})],$$

where *t* is time in days, *M* is gizzard mass in grams, α is the common intercept, $\beta_1$ and $\beta_2$ are the slopes of the first and second parts of the biphasic regression, respectively, *r* is a smoothness parameter that was set at 0.05, a rather abrupt transition, and γ is the breakpoint between the two parts of the biphasic regression. Since multiple solutions may occur, the continuous biphasic regression model was fitted several times through a data set using different initial values for α, $\beta_1$, $\beta_2$ and γ. The solution with the highest $r^2$ value was used for each data set. An exception was made for the switch from pellets to mussels in group B2, where the model yielded two 'best' solutions with almost equal $r^2$ values (0.3908 and 0.3955), but with very different rates of increase (0.31±0.06 and 0.92±0.26 g day$^{-1}$, respectively) and breakpoints (9.15±0.91 and 2.25±0.67 days, respectively). The solution with the lowest rate of increase and highest breakpoint seemed to describe the data best. The data for group C suggested that the gizzard started to increase in mass 2 days after the switch in food type so, in this case, we also tried to fit a triphasic continuous regression model. This extended version of the biphasic model did not add significantly to the explanation of the variance ($F_{2,94}=1.887$, $P>0.25$). We were unable to find statistical tools to correct for repeated sequential sampling of individuals.

Table 2. *Parameters of the continuous biphasic linear regression relationship between gizzard mass and the number of days after a switch from hard (mussels) to soft (pellet) food or vice versa*

| Diet switch | Group | N | Intercept (g) | Slope 1 (g day⁻¹) | Slope 2 (g day⁻¹) | Breakpoint (days) | P | $r^2$ | Initial mass (g) | Initial N | Final mass (g) | Final N | Relative change (% day⁻¹) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| From mussels to pellets | A | 80 | 8.02±0.23 | −0.46±0.05ᵃ | 0.17±0.11* | 8.50±0.76ᵃ | <0.0001 | 0.6522 | 8.33±0.31ᵃ | 10 | 4.73±0.19ᵃ·ᵇ | 30 | −5.1 |
| | B1 | 80 | 8.20±0.28 | −0.31±0.05ᵃ | 0.09±0.25* | 10.05±2.28ᵇ | <0.0001 | 0.4883 | 8.03±0.33ᵃ | 9 | 5.37±0.18ᵇ | 18 | −3.3 |
| | B2 | 84 | 9.71±0.33 | −0.40±0.09ᵃ | −0.06±0.29* | 6.58±2.40ᵃ·ᵇ | <0.0001 | 0.4507 | 6.59±0.42 | 9 | 3.89±0.29 | 18 | −6.2 |
| From pellets to mussels | A | 120 | 4.91±0.38ᵃ | 0.62±0.27ᵃ·ᵇ | −0.02±0.02* | 2.38±0.81 | <0.02 | 0.0877 | 4.91±0.32ᵃ | 10 | 6.18±0.13ᵃ | 100 | 10.9 |
| | B1 | 142 | 4.78±0.38ᵃ | 0.55±0.15ᵃ·ᵇ | −0.05±0.02 | 5.83±1.09ᵃ | <0.0001 | 0.3547 | 4.85±0.37ᵃ | 9 | 7.50±0.15ᵇ | 79 | 9.4 |
| | B2 | 72 | 4.66±0.29 | 0.31±0.06ᵃ | −0.41±0.26* | 9.15±0.91ᵇ | <0.0001 | 0.3908 | 4.13±0.33ᵃ | 9 | 6.78±0.19ᵃ·ᵇ | 18 | 7.0 |
| | C | 98 | 1.71±0.29 | 0.54±0.08ᵇ | 0.14±0.05 | 7.26±1.21ᵃ·ᵇ | <0.0001 | 0.7026 | 2.05±0.20 | 11 | 6.26±0.18ᵃ | 55 | 28.3 |

Values are given as means ± S.E.M.

Parameters indistinguishable from zero are indicated with an asterisk.

Within a column and diet switch, values with the same letters do not differ significantly from each other.

Also given are mean gizzard mass on the first day of the diet switch (initial mass) and mean gizzard mass at the plateau phase (final mass), as well as the relative change in gizzard mass over this period expressed as percentage of initial mass [100×(final mass−initial mass)/initial mass/breakpoint].

B1 (first trial) and B2 (second trial) indicate the two trials of group B.

Breakpoints did not differ significantly when they fell within the 95 % confidence intervals of each other. The slopes of the first part of the biphasic regression analysis (i.e. rates of increase or decrease in gizzard mass) were compared using the univariate analysis of variance procedure of SPSS. We used a Student's *t*-test to determine whether the rate of increase in estimated gizzard mass was equal to the rate of decrease (see Sokal and Rolf, 1997). Only if comparisons in either direction yielded a nonsignificant result did we conclude that the slopes were equal.

## Results

### General trends

After a switch from mussels to pellets or *vice versa*, red knots in groups A and B responded immediately with a decrease or increase in gizzard mass, respectively (Fig. 1). Body mass also varied with diet (Fig. 1), increasing after a switch from mussels to pellets and decreasing again when the birds were shifted to a diet of mussels. In three birds of group B, body mass started to increase rapidly from day 40 of the experiment (1 April) onwards, resulting in increases in the means and standard errors of group body mass. This may reflect mass gains related to northward migration that show up in captive birds (Piersma et al., 1995). The birds in group C had a delay of approximately 2 days before gizzard mass began to increase in response to the diet switch from trout pellets to mussels (Fig. 1). During this period, the birds ate little and body mass decreased by 10 g, i.e. 5 g day⁻¹.

The results show clearly that the effects of the food types on gizzard mass are reversible (Fig. 1). When birds with a decreased gizzard were fed a hard food type once more, their gizzard masses returned to the pre-soft food values measured earlier. Even in the birds of group C, which had been eating a soft food type for over a year, gizzard mass increased after the change to the hard food type to a size similar to that of the other groups. Repeated food type switching elicited repeated decreases and increases in gizzard mass (group B).

The responses of red knots to a switch in food type become more comparable when gizzard mass is plotted against time since the diet switch (Fig. 2). Continuous biphasic regression models were fitted through the data collected after each switch until the next switch. Table 2 gives the parameter values for these models.

### Switch from mussels to trout pellets

The rate of decrease in gizzard mass after a switch from mussels to trout pellets did not differ between the three trials (Fig. 2A; Table 2; N=140, F=1.997, P>0.1). The common rate of decrease in gizzard mass was −0.38±0.03 g day⁻¹. Initial gizzard mass did not differ between group A and the first trial of group B (B1), but was lower in the second trial of group B (B2; REGWQ-test). This difference was maintained after gizzard mass had levelled off. Final masses (averaged per trial over the plateau period) were lowest in group B2 and did not differ between groups A and B1 (Table 2; REGWQ-test). On

average, the gizzard decreased by 4.9 % day$^{-1}$ (expressed relative to initial mass) until it was 60.9 % of initial mass.

The variation around the estimate of the breakpoint of group A (i.e. the estimate of the start of the plateau phase) was small, and the breakpoint of group B1 (10.05 days) fell just outside the 95 % confidence interval of group A (6.98–10.02 days, Table 2). The breakpoints of groups B1 and B2 did not differ, however, and group A showed an intermediate value, so an overall mean breakpoint was calculated. Red knots reached a final reduction of 40 % in gizzard mass approximately 8.5 days after a switch from a hard to a soft food type.

### Switch from trout pellets to mussels

Initial gizzard masses at the switch from pellet to mussel food and the subsequent rates of increase in gizzard mass did not differ between groups A, B1 and B2 (Fig. 2B; Table 2; initial mass, REGWQ-test; slopes, $N=92$, $F=1.993$, $P>0.1$). The common rate of increase of these three trials,

0.30±0.05 g day$^{-1}$, was lower than the common rate of decrease in gizzard size after the switch from mussels to pellets (−0.38 g day$^{-1}$; Student's $t$-test, $P<0.05$).

Although group C started with a gizzard mass that was only 44 % of average gizzard mass in the other trials (REGWQ-test), final mass was similar to those of the other groups (Table 2). Also, the rate of increase in gizzard mass lay within the range of the other groups, although it differed significantly from that of group B2 (all groups, $N=135$, $F=2.589$, $P<0.05$; groups A, B1 and C, $N=90$, $F=0.095$, $P>0.9$, Table 2). Nevertheless, the calculated common rate of increase for all four trials, 0.42±0.04 g day$^{-1}$, was indistinguishable from the common rate of decrease (Student's $t$-test, $P>0.05$).

The relative rates of increase differed considerably between the freshly caught birds and the long-term captive birds, group C (Table 2). This was caused by the very low initial mass of group C (2.05±0.20 g) because the relative rate of increase was expressed relative to initial mass. The low initial mass also yielded a high relative final gizzard mass, more than 300 % of initial mass, while in the other groups it was only approximately 147 % of initial mass reached on average 6.2 days after the diet shift. Group C also showed a high relative rate of increase in gizzard mass: 28.3 % day$^{-1}$ *versus* on average 9.1 % day$^{-1}$ in the recently caught birds.
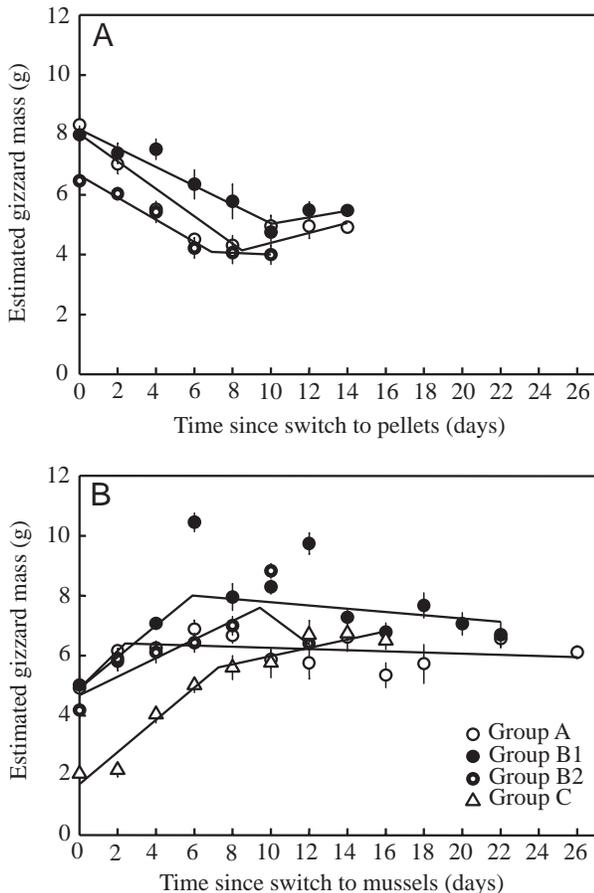


Fig. 2. Changes in gizzard mass after a diet switch from mussels to trout pellets (A) or after a switch from trout pellets to mussels (B). Lines indicate the results of the continuous biphasic regression model fitted through the data. Parameters of the biphasic regression models are given in Table 2. B1 represents the reaction of group B to the first diet switch trial; B2 represents the reaction of group B to the second diet switch trial. Values are means ± S.E.M.; group A ($N=10$), group B ($N=9$) and group C ($N=11$).

### Discussion
#### Time course and magnitude of changes

The gizzards of red knots needed more time to adjust during switches from a hard to a soft food type than *vice versa* (8.5 days *versus* 6.2 days for a switch from soft to hard). In contrast, Japanese quail needed slightly less time (5 days) when switching from a high- to a low-fibre diet than *vice versa* (6 days) (Starck, 1999a). Nevertheless, the times necessary for change were rather similar for the molluscivorous knot and the granivorous quail, as were the relative magnitudes of the decreases in gizzard mass (approximately 61 % of initial mass in both species).

The extent of upregulation of gizzard mass in quail (211 % of initial mass; Starck, 1999a) was half-way between that of the recently caught red knots (147 %) and the long-term captive birds (305 %). In both the quail and the long-term captive knots, the gizzards were initially rather small because of past long-term eating of a soft food type (i.e. a low-fibre diet in quail and pellets in red knots). If we reanalyse the quail data, using as the initial value for gizzard mass that for birds recently shifted to a low-fibre diet, the increase in gizzard mass changed to 163 % of initial mass, which is rather similar to that in freshly caught knots.

#### Limitations and caveats

The response to a shift in diet to pellets was similar in all the groups (Fig. 2A). The mussel diet elicited a more variably timed increase in gizzard mass (Fig. 2B). This may have been due to variability in the mussels. Unlike the pellet diet, which was of constant quality, the mussel diet consisted of naturally

available mussels that, by necessity, varied somewhat in size. They also varied in quality (meat/shell mass ratio; see Dekinga and Piersma, 1993) between, and probably also within, size classes. During the experiments, the mussels on offer became progressively smaller as 1-year-old mussels became too big and more young mussel spat became available. Smaller mussels had higher meat/shell mass ratios, so the red knots may have eaten these mussels first. This factor was not quantified.

### Pure use/disuse mechanism?

The digestive tract of constrictors, such as the Burmese python *Python molurus*, increases enormously in size immediately after eating a meal, the change being most pronounced in the intestines (an approximately 150 % increase in the course of a single day; Secor and Diamond, 1998). This increase takes place before the intestines are involved in the digestive process, so that mechanisms other than a use/disuse mechanism must be responsible for their change in size (Piersma and Lindström, 1997; Starck and Beese, 2001).

The gizzards of knots kept on trout pellets for at least a year (2.05 g, initial gizzard mass of group C) were only half the size of the gizzards of knots recently exposed to this soft food type (4.63 g, mean final gizzard masses of groups A, B1 and B2, Table 2). In an earlier non-invasive study, we followed changes in gizzard mass in red knots that had been shifted from shellfish to pellets for approximately a year (Dietz et al., 1999b): 86 days after the switch, gizzard size had been reduced to approximately 54 % of initial mass. This suggests that the freshly adjusted small gizzard size was maintained for at least 80 days. After 260 days on a pellet diet, gizzard mass had reached a final value of 24 % of initial mass. Thus, gizzard mass must have decreased further, and the immediate response to a switch from a hard to a soft food type documented here is partial. This implies that there is more to the diet-related decrease in gizzard mass in red knots than 'use/disuse' alone.

This is confirmed by the observation that the gizzard size of Japanese quail did not return to low pre-experimental values within 14 days (Starck, 1999a). Starck (Starck, 1999a) seeks explanations for this phenomenon in two types of constraint. There could be a mechanical constraint, i.e. a differential downregulation of the various tissue types of the gizzard, or, more likely, there could be a strategic constraint, i.e. the gizzard is downregulated to a level at which the possibilities of coping with another challenge and the costs of maintenance are balanced. This may be an 'insurance principle' commonly used to cope with a decrease in challenge or workload, similar to the type of response found in sponges (the demosponge *Halichondria panicea*) transplanted between environments with low and high wave energy (Palumbi, 1984). Within 4 weeks after transplantation of sponge parts from a site of low to a site of high wave energy, the newly grown skeleton was just as stiff and strong as that of sponges long present in a high-energy wave environment. In contrast, after transplantation from a high to a site of low wave energy, it took the sponges 10 weeks before their newly grown skeleton decreased in stiffness and strength. As Palumbi (Palumbi, 1984) indicates,

one would expect a delayed or partial response only if the risks of maintaining inappropriate morphologies are asymmetric. In places with heavy wave action, a soft skeleton is risky, whereas a stiff skeleton at sites of low wave action induces no extra risks. Similarly, for red knots, a small gizzard represents a risk when suddenly only hard-shelled food can be found.

Even after a long period on a soft diet, the recovery capacity of the gizzard of red knots did not seem to be compromised because the rate of increase in the long-term captivity group (group C) was similar to that of the recently caught birds (groups A, B1 and B2, Table 2). Nevertheless, these birds experienced serious problems with the switch because body mass dropped by 10 g in 2 days. It then took the birds 10 days to restore their body mass to the initial level.

### Ecological implications

We propose that gizzard size adjustments are exquisitely finely tuned to the particular ecological conditions. During the 50 day breeding season on the high arctic tundra, red knots feed mainly on soft-bodied surface arthropods and spiders, so they should arrive south with small gizzards. Although they could then switch to a shellfish diet, this would compromise their capacity to maintain body mass immediately after arrival. Red knots may arrive with rather low body masses in the Wadden Sea in July and August (between 90 and 120 g; Dick et al., 1986; Gromadzka, 1990; Nebel et al., 2000), so a further decrease could be life-threatening (Piersma, 1994).

In July 1994, 14 red knots *C. c. islandica* using southwest Iceland as a first stopover site on their return from the high arctic breeding grounds were examined (Piersma et al., 1999b). On the basis of the variation in body mass (T. Piersma, G. A. Gudmundsson and K. Lilliendahl, personal communication), four of these red knots had apparently just arrived from the breeding grounds, while the other 10 birds may have been feeding in the area for a while (average body masses were $119.5\pm4.8$ g, $N=4$, and $141.8\pm8.9$ g, $N=10$; $F=21.786$, $P<0.01$). The birds that had just arrived had smaller gizzards than the other 10 birds ($4.77\pm0.49$ g, $N=4$, *versus* $6.22\pm0.81$ g, $N=10$; $F=10.929$, $P<0.01$). The gizzard mass of the recently arrived birds fell within range of the average final masses of our experimental birds after the switch from mussels to pellets (groups A, B1 and B2; range 4.13–4.91 g, Table 2) and was more than twice the average initial gizzard mass of the long-term captives (group C, 2.05 g).

Thus, red knots recently arrived from the tundra breeding grounds (where they ate soft-bodied invertebrates) have gizzards larger than expected on the basis of the long-term atrophy patterns documented for captive birds eating soft food (Dietz et al., 1999b). Such birds have either 'defended' gizzard size during the breeding season or even strategically increased gizzard size before southward departure. The costs of such proactive responses (e.g. the extra cost of carrying additional structure in flight) must be outweighed by the benefits upon arrival at stopover sites. Putting together such a cost/benefit analysis for organ size changes remains a real challenge in evolutionary physiology.

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