

## RESEARCH ARTICLE

# Red knots (*Calidris canutus islandica*) manage body mass with dieting and activity

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## ABSTRACT

Mass regulation in birds is well documented. For example, birds can increase body mass in response to lower availability and/or predictability of food and decrease body mass in response to increased predation danger. Birds also demonstrate an ability to maintain body mass across a range of food qualities. Although the adaptive significance of mass regulation has received a great deal of theoretical and empirical attention, the mechanisms by which birds achieve this have not. Several non-exclusive mechanisms could facilitate mass regulation in birds. Birds could regulate body mass by adjusting food intake (dieting), activity, baseline energetic requirements (basal metabolic rate), mitochondrial efficiency or assimilation efficiency. Here, we present the results of two experiments in captive red knots (*Calidris canutus islandica*) that assess three of these proposed mechanisms: dieting, activity and up- and down-regulation of metabolic rate. In the first experiment, knots were exposed to cues of predation risk that led them to exhibit presumably adaptive mass loss. In the second experiment, knots maintained constant body mass despite being fed alternating high- and low-quality diets. In both experiments, regulation of body mass was achieved through a combination of changes in food intake and activity. Both experiments also provide some evidence for a role of metabolic adjustments. Taken together, these two experiments demonstrate that fine-scale management of body mass in knots is achieved through multiple mechanisms acting simultaneously.

**KEY WORDS:** Activity, Diet, Food quality, Mass loss, Mass regulation in birds

## INTRODUCTION

The ability to regulate body mass in the face of changing conditions has major functional importance for birds. Both theoretical (Bednekoff et al., 1994; Bednekoff and Houston, 1994; Houston and McNamara, 1993; Lima, 1986) and empirical work (Bednekoff and Krebs, 1995; Cuthill et al., 2000; Ekman and Hake, 1990) have shown that increasing fat stores is an adaptive response to decreased availability or predictability of food. However, carrying fat stores

also incurs costs. The metabolic cost of activities increases with increasing body mass (Nagy, 2005; Song and Beissinger, 2020), and flight performance may be especially compromised (Bednekoff, 1996; Houston and McNamara, 1993; Kvist et al., 2001; Pennycuik, 1989). All else being equal, carrying more fat should decrease take-off angle and speed, which can reduce the ability to evade predators (Pennycuik, 1989). However, scenarios of ‘all else being equal’ may not be the norm. For example, many studies show that birds maintain near constant flight performance under changing body mass by invoking compensatory mechanisms such as adjustment in pectoral muscle mass or changes in flight efficiency (Dietz et al., 2007; Kvist et al., 2001; van der Veen and Lindström, 2000; Walters et al., 2017). Nonetheless, as long as these compensatory mechanisms incur costs, it remains that birds must balance the survival benefit of carrying more fat via its effect on the probability of starvation, against the cost of fat on escape flight performance (McNamara and Houston, 1990). Such costs may either be direct survival costs or costs incurred to evoke compensatory mechanisms.

Indeed, birds show an exceptional capacity to fine-tune their body mass in response to variation in the availability and/or predictability of food and variation in predation danger. For example, within a single day, birds can increase body mass by up to 10% between dawn and dusk, with fat stores accumulated over the foraging period being subsequently lost during overnight fasting (Lilliendahl, 2002; MacLeod et al., 2005; Moiron et al., 2018; Thomas, 2000). Furthermore, the timing of mass gain during the day depends on the predictability of access to food and the local predator landscape (Bednekoff and Houston, 1994). Under elevated predation danger, birds delay mass gain until later in the foraging period (McNamara et al., 1994).

Birds also regulate body mass over longer time scales. For example, across the annual cycle, individual body mass may vary more than twofold in relation to migration stage (Karagicheva et al., 2016; Piersma et al., 2008; Piersma and Gill, 1998; Piersma and Jukema, 2002). Body mass can also vary over much longer time scales, such as across generations. For example, western sandpipers (*Calidris mauri*) at stopover sites on migration (Ydenberg et al., 2004) and Eurasian golden plovers (*Pluvialis apricaria*) on autumn staging grounds (Piersma et al., 2003b) have both shown decreases in body mass of approximately 10% over a period of 20 years. These multi-generation shifts are thought to be responses to changes in the predator landscape as they coincide with the recovery of populations of a major avian predator, peregrine falcons (*Falco peregrinus*).

Even in long-term captive studies, birds continue to exhibit strong body mass regulation that reflects the trade-off between the costs and benefits of carrying extra fat. Captive birds exhibit circadian (Dall and Witter, 1998) and circannual patterns (Karagicheva et al., 2016) of body mass, and adjust their body mass in response to variation in perceived predation risk (van den Hout et al., 2010) and experimental manipulations of food availability (Cuthill et al.,

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2000; Ekman and Hake, 1990). Even under conditions of unrestricted access to food, unlike mammals, birds rarely become ‘overweight’ (Halsey, 2018). The adaptive significance of mass regulation in birds has received a great deal of attention in both theoretical and empirical studies, although the mechanisms by which birds regulate their body mass have not (Halsey, 2018; but see Kelly and Weathers, 2002).

Birds could regulate their body mass using several non-exclusive mechanisms (reviewed in Halsey, 2018). All else being equal, mass loss could be facilitated by decreased food intake, increased activity, higher metabolic rate, lower mitochondrial efficiency or lower digestive efficiency. Here, we present the results of two experiments to evaluate the relative importance of three of these mechanisms for mass regulation in red knots (*Calidris canutus islandica*). In the first experiment, knots exhibited presumably adaptive changes in body mass in response to perceived predation risk (Mathot et al., 2009; van den Hout et al., 2010). We use activity budget observations to evaluate whether predation-related decreases in body mass were driven by decreased food intake, increased activity, or both. In the second experiment, knots maintained their body mass across large experimental manipulations in the quality of their diet (Mathot et al., 2019). We use activity budget observations and respirometry data to evaluate whether variation in diet quality was associated with changes in food intake, activity, basal metabolic rate (BMR) or a combination of the three. Taken together, our results provide evidence that both dieting and activity are key mechanisms underlying mass regulation in captive red knots, but also hint at a potential role of metabolic adjustments.

## MATERIALS AND METHODS

### Study species

Red knots (*Calidris canutus*) are medium-sized migratory shorebirds, and are ideally suited for studies of the mechanisms of mass regulation (Karagicheva et al., 2016). Previous work in red knots has shown that they exhibit adaptive changes in body mass in response to manipulations of perceived predation danger (Mathot et al., 2009; van den Hout et al., 2010), and that they maintain regular seasonal variation in body mass even after extended periods of time in captivity (Karagicheva et al., 2016).

The experiments complied with Dutch law regarding animal experiments (Dutch Animal Ethics Committee Licences NIOZ 04.04 and NIOZ 10.05 addendum 5, and protocol no. AVD802002016740).

### Experiment 1: adaptive changes in body mass in response to perceived predation danger

#### Study subjects

Fifty knots of the *Calidris canutus islandica* subspecies (Linnaeus 1767) were captured using mist nets on the mudflats of Richel (53° 16′ 57″ N, 05° 23′ 82″ E) and Simonszand (53° 29′ 28″ N 06° 24′ 19″ E) in the Wadden Sea, The Netherlands, on 8 August and 3 September 2005. Prior to experiments, birds were housed at the NIOZ (Nederlands Instituut voor Zeeonderzoek) Royal Netherlands Institute for Sea Research in outdoor aviaries and given an *ad libitum* diet of mudsnail, *Peringia ulvae* (formerly *Hydrobia ulvae*).

#### Experimental procedure

Experiments were carried out between 21 August and 28 November 2005. Birds were randomly assigned to flocks, with eight flocks of six birds being tested ( $N=48$  birds). Experiments were conducted in the indoor experimental shorebird facility at NIOZ (7.0 m × 7.0 m × 2.8 m high), under constant photoperiods with

lights on from 06.00 to 21.00 h, and ‘moonlight’ mimicking illumination being provided during the dark phase. Flocks were tested sequentially. After being introduced to the mudflat facility, the focal flock was given 2 days to acclimate to the mudflat before the start of experiments, which consisted of a 5-day control treatment (no disturbance) and a 5-day experimental period (exposure to model predator). Treatment order was randomized, with half of the flocks receiving the control treatment first, and half of the flocks receiving the experimental treatment first. During the experimental treatment, flocks were subjected to two manipulations of perceived predation each day: one presentation of a gliding sparrowhawk (*Accipiter nisus*), and one presentation of a perching sparrowhawk. The timing and order of events was randomized, and behavioural observations were conducted before and after each predator exposure. The control period consisted of no disturbances, but observation periods were time-matched to the disturbances during the treatment periods to control for temporal variation in activity budgets.

#### Activity budget observations

During both the control and experimental phases of the experiment, 5 min continuous, focal observations were made on each flock member four times each day, before and after each of the two simulated predator encounters. For the control phase, there were no simulated predator encounters, and observations were made at time-matched periods to those of the predator phase. Individual observation order was randomized. Observations were conducted from behind a one-way mirror, dictated into a microcassette recorder, and later transcribed using The Observer 3.0 Event Recorder (Noldus Information Technology, Wageningen, The Netherlands). All observations were made by a single observer (K.J.M.).

During focal observations, we noted both feeding and activity. Feeding was defined as the focal bird being present in the food tray and pecking at the food. Although we could not evaluate individual intake rates, we have previously shown that treatment-related differences in proportion of time spent foraging coincide with treatment-related differences in food intake; when knots spend less time ‘feeding’, less food is consumed (Mathot et al., 2009).

We used movement in a familiar environment as our measure of activity (*sensu* Réale et al., 2007). In this experiment, ‘activity’ consisted of walking with the head oriented upwards, as well as walking with the head oriented downwards, including walking while pecking at the substrate as long as this was not within the food tray (which was scored as ‘feeding’). Although ‘flying’ would also be considered an active or movement-generating behaviour, it was not observed during any of the focal observations included in this analysis, and thus did not contribute overall to our estimate of proportion of time spent active. Additional behaviours were scored including vigilance (standing still with the bill parallel to the horizon or higher with accompanying side-to-side movement of the head), resting (motionless with the head up), roosting (motionless with bill tucked into a wing), and preening (all behaviours related to plumage maintenance). However, as these behaviours are not assumed to be indicative of feeding or activity, they are not discussed further.

#### Data selection and statistical analysis

We previously showed that knots adjusted body mass in response to manipulations of perceived predation danger as expected from theory; at the population level, body mass was lower following the

predator treatment compared with the control treatment (van den Hout et al., 2010). We also previously showed that at the population level, knots showed dramatic short-term behavioural responses to perceived predation danger by decreasing their investment in foraging immediately following experimental presentations of predator models (Mathot et al., 2009). Here we ask how much of the treatment-related changes in body mass can be explained by long-term behavioural adjustments of foraging and activity. We do this in two steps. First, we evaluate long-term responses to the predator treatment relative to control in terms of (1) mass trajectories, (2) proportion of time spent foraging and (3) proportion of time spent active. To do this, we constructed three separate linear mixed-effect models using the ‘lmer’ function in R (<https://github.com/lme4/lme4/>) with treatment (predator or control) as a fixed effect. We included individual and flock identity (ID) as random effects to account for non-independence of repeated measures on the same individuals, and on individuals within the same flock, respectively. Because we were explicitly interested in long-term responses, we estimated mass trajectories as the change in body mass across each 5-day treatment period (end body mass minus starting body mass, in grams) such that negative values within a given treatment reflected mass loss, and positive values within a given treatment reflected mass gain. Because the experimental design included measures of behaviour both before and after presentation of sparrowhawk models, repeated over 5 days, we captured both short- and long-term behavioural responses to manipulations of perceived predation danger. For a schematic of the experimental set-up and the data included in this study, see Fig. S1. As we were explicitly interested in the long-term response to manipulations of perceived predation danger, we chose to use the first daily behavioural observation period from days 2 to 5 of each treatment block because these captured long-term changes in behaviour (i.e. carried over from the previous day). We did not include day 1 of each treatment block, as knots had not yet experienced a predator treatment at the start of day 1. Estimates of time spent foraging within each treatment period was done by summing the proportion of time spent feeding across each observation and dividing by the number of observations ( $N=4$ ). The same was done for time spent active.

Next, we asked whether within-individual changes in mass trajectory across the two treatments were predicted by within-individual changes in time spent foraging and/or time spent active. We calculated the difference between treatments as the average proportion of time feeding during the predator treatment minus the average proportion of time spent feeding during the control treatment, such that negative values reflect relatively lower feeding during the predator compared with the control treatment. The same was done for proportion of time active (i.e. walking when not feeding). We constructed a linear mixed-effect model to test whether within-individual changes in mass trajectory (dependent variable) were predicted by within-individual changes in time spent foraging (independent variable) or within-individual changes in time spent active. Each individual only appeared in the data set once, so we did not model individual ID. However, we included flock ID as a random effect to account for non-independence among flock members.

For all models, we used the ‘sim’ function of the ‘arm’ package to simulate the posterior distribution of the model parameters. Ninety-five per cent credible intervals (95% CrI) around the mean ( $\beta$ ) were extracted based on 1000 simulations (Gelman and Hill, 2007). Effects were considered significant when the 95% CrI did not overlap 0. Estimates whose credible intervals were biased away

from 0, but whose credible intervals overlapped 0 by up to 15%, were interpreted as showing moderate support for an effect, as these provided more than five times greater support for the interpretation of an effect than they did for the interpretation of no effect (Cumming and Finch, 2005). Bayesian  $P$ -values were calculated when estimates were biased away from 0 but 95% CrI overlapped 0. Adjusted among-flock repeatability was calculated following Nakagawa and Schielzeth (2010).

## Experiment 2: maintaining body mass under changing diet quality

### Study subjects

Eighty-four red knots were captured using mist nests on the mudflats of Richel (53°16'N, 05°23'E), Griend (53°14'N, 05°15'E) and Schiermonnikoog (53°28'N, 06°10'E) in the Wadden Sea, The Netherlands, between August and October 2015 and transported to the experimental shorebird facilities at the NIOZ. Birds were housed in eight outdoor aviaries (4 m deep×1.9 m wide×2.3 m high at one end, sloping down to a height of 1.9 m at the other end) in flocks of between 14 and 17 individuals (including individuals of the *C. c. islandica* subspecies that were part of another study). Knots were maintained on an *ad libitum* diet of protein-rich trout pellets (Trouvit, Produits Trouw, Vervins, France). Birds were handled each week to assess overall health (Milot et al., 2014) and to allow the aviaries to be cleaned. Further details on husbandry conditions are provided in Mathot et al. (2019).

### Experimental procedure

Experiments were conducted over two years between October 2015 and March 2017. Data collection was restricted to between October and March in each year. This is outside the migration period of the *islandica* red knots (Dietz et al., 2007), and thus knots were expected to exhibit relatively little adaptive seasonal variation in body mass during this time. Birds experienced four diet manipulations in each year (eight in total). Diets alternated between high digestive quality fish pellets (Trouvit; the same high digestive quality food provided outside the experimental period) and low digestive quality mudsnails (*Peringia ulvae*). Mudsnails are low digestive quality because they must be consumed whole, including the indigestible shell. As a result, Trouvit has a ~4-fold higher energy density (17.66 kJ g<sup>-1</sup>, value from manufacturer) compared with *P. ulvae* (4 kJ g<sup>-1</sup>) (van Gils et al., 2005a). The diet manipulations were used to induce variation in gizzard mass as part of another study (Kok et al., 2019; Mathot et al., 2019). Diet manipulations explained <1% of the observed variation in overall body mass (Mathot et al., 2019), indicating that knots on average maintained similar body mass across diet manipulations. In this study, we follow up on that finding to ask what are the relative contributions of changes in time spent foraging, activity and metabolism in allowing for this diet-independence of body mass. Body mass and BMR were measured for up to four birds per night at the end of each staple diet period (see below) in both years ( $N=80$ ). However, activity budget scoring was only implemented during the staple diet periods in blocks 2, 3 and 4 of year 2 ( $N=59$  birds).

### Activity budget observations

Behavioural observations were conducted on birds in their home aviary beginning in year 2 of the study, and were carried out in blocks 2, 3 and 4 of that year. Observations were conducted during three time blocks: morning (11.00–12.00 h), mid-day (13.40–14.40 h) and afternoon (15.30–16.30 h). Observers ( $N=4$ ) were



randomly assigned to an aviary ( $N=8$ ) on any given observation session, and observations were made through a one-way mirror in the aviary door. All birds present in the aviary were assigned a random observation order. Prior to commencing observations, the observer would record the aviary and the focal bird in JWatcher (<http://www.jwatcher.ucla.edu/index.html>). Next, they would scan the flock until the randomly assigned focal bird was located. Once the bird was located, the observation began for 5 min. If the randomly assigned bird could not be located within 1 min, the observer moved on to the next bird on the list, and the missed bird was attempted again at the end of the observation session. This could occur, for example, if several birds, including the focal bird, were in the roosting posture with one leg tucked under the belly, preventing reading of the full combination of leg bands.

During observations, the following behaviours were recorded: walking, flying, resting, roosting, vigilance, preening, stretching, ruffle, eating, drinking, pecking, searching, defaecating, bathing, vocalizing, social interactions, miscellaneous and out of sight. The ethogram with a description of each behaviour are provided in Table S1. As with experiment 1, we focused on changes in foraging and activity as potential mechanism of body mass regulation. Similar to experiment 1, 'activity' was defined as the sum of the proportion of time engaged in movement behaviours. In experiment 2, this included time spent walking, flying and searching. This differs slightly from experiment 1 in that no flying was observed in experiment 1, so it was not included in the time spent active total in experiment 1. However, in experiment 2, proportion of time spent flying made up a very small amount of the total activity budget (mean 0.06%, range 0.00 to 0.70%). As such, including it neither qualitatively nor quantitatively changed any of the results discussed (analyses not shown). However, as we *a priori* considered flying an active behaviour, we present analyses including it in the main text. Although observations were meant to be 5 min each, a number of observations terminated early when the bird was lost from view. Proportion of time spent active and proportion of time spent feeding were calculated based on the total observed duration of the relevant behaviours divided by the total observation duration (i.e. excluding time when bird was out of sight).

### Basal metabolic rate measurements

BMR was measured using flow-through respirometry to evaluate whether BMR was adjusted in response to changes in diet quality. Knots were allowed 3 weeks of adjustment to their current diet before BMR measurements were taken, to ensure that birds had sufficient time to physiologically adjust to diet quality. We were able to measure BMR for three birds per night in the first year, and four birds per night in the second year because we obtained an additional mass flow controller in year 2. The order in which BMR was recorded for each bird was randomly determined. BMR measurements occurred 1 day after birds were subjected to a 30 min behavioural observation in a novel environment to score exploration as part of another study (Kok et al., 2019). On the day that an individual was scheduled to have their BMR recorded, individuals were removed from their home aviary at approximately 09.00 h and placed in a holding crate without food until BMR measurements commenced later that afternoon. This duration of food deprivation was necessary to ensure that knots had attained a post-absorptive state prior to the start of metabolic rate measurements as red knots have naturally cyclical foraging routines and may require several hours to clear their digestive tracts. In the wild, knots undergo natural fasts that coincide with the tidal cycle; they do not forage during high tides when their foraging grounds are inundated, and

forage intensively during low tides (van Gils et al., 2005b; van Gils et al., 2006). At 16.00 h, birds were weighed to the nearest 0.1 g.

Next, birds were put into individual air-tight metabolic chambers (6.8 liters) that were placed within an environmental cabinet (model HETK 3057.S, Weiss Enet, Wijk bij Duurstede, The Netherlands). The environmental cabinet was kept at 21°C, which is within the thermoneutral zone of red knots (Piersma et al., 1995; Wiersma and Piersma, 1994). Outdoor air was dried (Drypoint membrane dryer model 1201, Beko, Redditch, UK) before being pumped through each chamber at a rate of 50 ml min<sup>-1</sup>. Flow rates were measured by mass flow controllers (model 5850Z, Brooks Instruments, Venendaal, The Netherlands), which were calibrated using a Bubble-O-Meter (Dublin, OH, USA). Effluent air was scrubbed of H<sub>2</sub>O using a molecular sieve (2 mm granules, Merck, Darmstadt, Germany). The O<sub>2</sub> and CO<sub>2</sub> concentrations of influent and effluent airstreams were measured using O<sub>2</sub> (model 4100, Servomex, Zoetermeer, The Netherlands) and CO<sub>2</sub> (model 1400, Servomex) analysers. An automatic valve switched between airstreams so that 10 min of baseline was recorded followed by 30 min of effluent air from each of two chambers (60 min total). This sequence was repeated throughout the 16 h measurement period for each of two analysers, allowing up to four birds to be measured per night.

Analysers were calibrated daily. Pure nitrogen was used as the low reference for both the O<sub>2</sub> and the CO<sub>2</sub> analysers. A standard gas of 0.499% CO<sub>2</sub> was used as the high reference for the CO<sub>2</sub> analyser, and dry air (assumed to contain 20.95% O<sub>2</sub>) was used as the high reference for the O<sub>2</sub> analyser. The following morning at ~08.00 h, birds were removed from the chamber, weighed and scored for subcutaneous fat before being returned to their home aviaries with *ad libitum* access to food and water.  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$  were calculated using the equations provided in Piersma et al. (2004). The lowest 10 min run of  $\dot{V}_{O_2}$  was used as our measure of BMR. Calculations were performed using ExpeData (Sable Systems, Las Vegas, NV, USA).

### Data selection and statistical analysis

Using data collected in the same set of experiments, we previously showed that body mass did not vary substantively as a function of diet treatment, but gizzard mass and pectoral muscle mass did (Kok et al., 2019; Mathot et al., 2019). Here, we present analyses on previously unpublished activity budget data and metabolic rate data.

To evaluate diet-related differences in body mass ( $N=80$  individuals), foraging ( $N=53$  individuals), activity ( $N=53$  individuals) and metabolic rate ( $N=80$  individuals), we constructed four separate linear mixed effect model using the 'lme' function of the 'lmer' package in the R statistical environment. Sample sizes for behavioural data were lower than for body mass and BMR data because focal observations were only initiated in year 2, and some birds had been removed from the study either due to *Staphylococcus* infections, or to make room for new birds that were brought into the experimental shorebird facility (Kok et al., 2019). For these univariate models, we included diet as a fixed effect, and individual and aviary as random effects. For analyses of metabolic rates, we included the additional random effect of chamber ID to account for possible analyser and/or chamber-related effects which would have introduced non-biological noise to the data. Inclusion of this random effect did not alter our interpretation of the effect of diet, but reduced the model residual variance (analyses not shown). We did not include date as a fixed effect in the model because our study design was balanced. During any given observation session, half the birds received the high-quality food treatment and half received the low-quality food treatment. Thus, treatment and seasonal effects (e.g. photoperiod) were not confounded, although we accounted for potential seasonal variation by including

**Table 1. Experiment 1: treatment-related differences in body mass trajectories, proportion of time spent feeding and proportion of time spent active in knots exposed to experimental manipulations of perceived predation risk**

	Mass trajectory	ln (Time feeding)	ln (Time active)
Fixed effects	$\beta$ (95% CrI)	$\beta$ (95% CrI)	$\beta$ (95% CrI)
Intercept	4.66 (2.55, 6.15)	-2.32 (-2.75, -2.02)	-2.20 (-2.28, -1.75)
Predator treatment	-6.26 (-7.67, -3.52)	-0.44 (-0.70, -0.08)	-0.48 (-0.74, 0.22)
Random effects	$\sigma^2$ (95% CrI)	$\sigma^2$ (95% CrI)	$\sigma^2$ (95% CrI)
Bird ID	$1.18 \times 10^{-14}$ ( $7.11 \times 10^{-15}$ , $1.88 \times 10^{-14}$ )	0.023 (0.016, 0.041)	0.07 (0.04, 0.10)
Flock ID	1.94 (0.33, 6.76)	0.16 (0.06, 0.49)	0.63 (0.18, 1.23)
Residual	31.46 (21.55, 38.45)	0.60 (0.49, 0.85)	0.41 (0.31, 0.55)

Foraging and activity data were ln-transformed prior to analyses to meet assumptions of normality for model residuals. The intercept estimate is for the control treatment.

'block' as a random effect. Proportion of time spent foraging and proportion of time active were log ( $n+0.01$ ) transformed prior to analyses. We used the 'sim' function of the 'arm' package (<https://CRAN.R-project.org/package=arm>) to simulate the posterior distribution of the model parameters. Ninety-five per cent credible intervals (95% CrI) around the mean ( $\beta$ ) were extracted based on 1000 simulations (Gelman and Hill, 2007). Effects were interpreted in the same way as for experiment 1 based on the effect size and the degree of overlap between the 95% CrI and 0. Adjusted repeatability was calculated following Nakagawa and Schielzeth (2010).

### Post hoc analyses

In both experiments, mass regulation was achieved through changes in the proportion of time spent feeding and changes in the proportion of time spent active (see Results). Coincident increases in activity with decreases in feeding could be due to the fact that proportions are bounded between 0 and 1, and that increases in one force a concomitant decrease in the other. If that was the case, then shifts in feeding should be directly offset by shifts in activity (i.e. the within-individual correlation should be  $r=-1$ ). Within-individual correlations of  $0 > r > -1$  would indicate that there was scope for partial independence in the investment towards these two components of the total activity budget, while a correlation of  $r=0$  would be indicative of complete independence.

To evaluate the scope for independent regulation of activity and feeding, we constructed bivariate mixed-effects models using the 'MCMCglmm' function (<https://CRAN.R-project.org/package=MCMCglmm>) in the R statistical environment. The proportion of time spent active and the proportion of time spent feeding (both natural log+0.01 transformed) were fitted as the response variables, and we modelled a random intercept for individual identity. We did not include additional random effects because none of the effects that were found to be important varied within individuals (Tables 1 and 3), and therefore would not influence our ability to estimate the within-individual correlation.

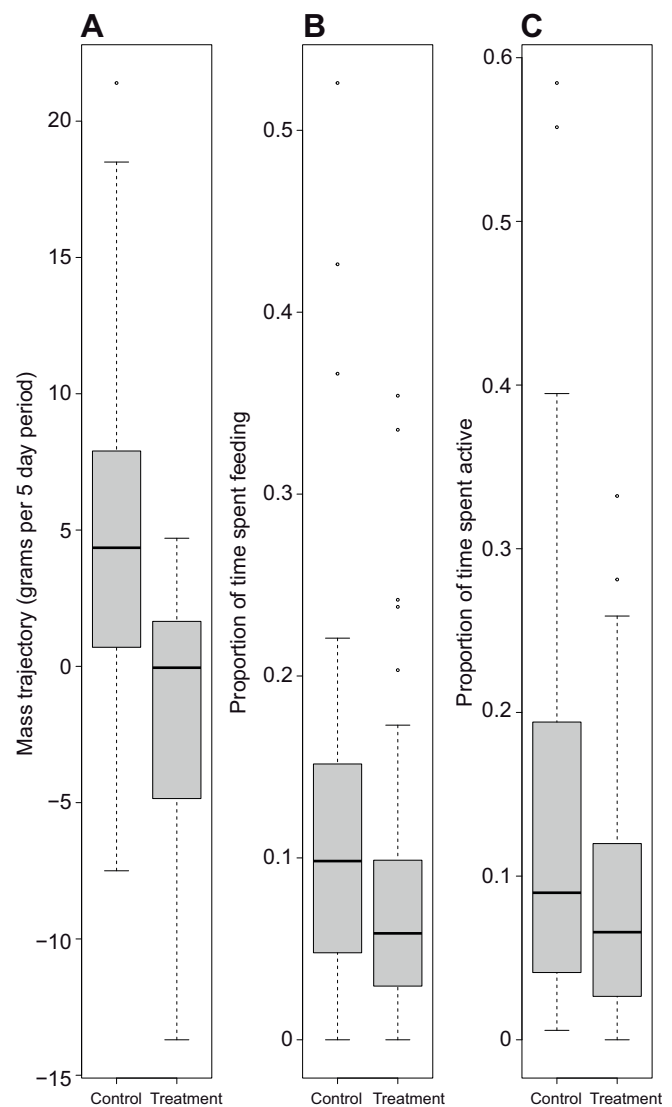
Results presented use an inverse gamma prior. However, we confirmed that the prior specification did not unduly influence the results. Results were quantitatively similar when using an inverse Wishart prior, and were not sensitive to nu (degrees of belief in the prior). We present estimates obtained when nu=2. Models were run for 103,000 iterations (i.e. nitt), a burn-in period of 3000, and a thinning interval of 100 to produce a total of 1000 estimates. The estimates were used to calculate the posterior mode of the distribution (i.e. the most likely value), as well as the 95% credible interval.

## RESULTS

### Experiment 1

Knots had significantly lower mass trajectories during the predator treatment compared with the control treatment, and spent

significantly less time foraging and less time active (Table 1; Fig. 1). At the within-individual level, increased investment in feeding was associated with significantly more mass gain ( $\beta=19.98$ ; 95% CrI 7.20, 43.60) (Table 2). That is, for every 0.1 increase in the



**Fig. 1. Treatment-related differences in mass trajectory and proportion of time spent feeding or active in red knots (*Calidris canutus islandica*).** Results from Experiment 1. Boxplots illustrate median (center line), upper and lower quartiles (upper and lower edges of box) and  $1.5 \times$  interquartile range (whiskers). The same individuals ( $N=48$ ) were included in both 'control' and 'predator' treatments.

**Table 2. Experiment 1: sources of variation in relative mass trajectory and relative activity trajectory in red knots exposed to manipulation of perceived predation danger**

Fixed effect	Delta mass trajectory $\beta$ (95% CrI)
Intercept*	-7.75 (-11.95, 0.34)
Change in proportion of time spent foraging <sup>†</sup>	25.97 (7.71, 45.96)
Change in proportion of time spent active <sup>‡</sup>	-22.49 (-56.83, 17.27)
Random effects	$\sigma^2$ (95% CrI)
Flock ID ( $N=8$ levels)	73.31 (46.14, 96.39)
Residual ( $N=48$ levels)	27.00 (17.37, 41.25)

Relative mass trajectory refers to the mass trajectory during the 5-day predator treatment minus the mass trajectory during the control treatment, such that negative values indicate a relative decrease in mass during the predator treatment. \*Intercept estimates the average change in body mass trajectory in perceived predation periods relative to control periods for individuals that show no adjustment in the proportion of time spent feeding. <sup>†</sup>Change in proportion of time spent feeding/active in predator treatment relative to control, where zero equals no net change, positive values indicate increase in time allocation during predator treatment, and negative values indicate decrease in time allocation in predator treatment.

proportion of time spent feeding during the predator treatment, knots gained an average of 2.00 g (Table 2, Fig. 2A). In contrast, increased investment in activity was associated with lower rates of mass gain ( $\beta=-22.93$ ; 95% CrI -56.05, 20.53; Bayesian  $P$ -value=0.15) (Table 2). That is, for every 0.1 increase in the proportion of time spent active during the predator treatment, knots gained on average 2.29 g less than the mean weight gain (Table 2, Fig. 2B). Furthermore, our multivariate analyses revealed that increasing activity did not force a concomitant decrease in feeding, or vice versa, as the estimated within-individual correlation from the bivariate model was 0.48 (95% CrI 0.27, 0.66). However, even for knots that showed no net change in proportion of time feeding or proportion of time active, there was moderate support that exposure to predators resulted in a decrease in body mass trajectory (intercept estimate:  $\beta=-3.81$  g; 95% CrI -11.40, 1.00; Bayesian  $P$ -value=0.04; Table 2).

## Experiment 2

There was moderate support for body mass varying with diet when analysing the subset of birds ( $N=59$  individuals) for which we had BMR data available (Table 3, Fig. 3A;  $\beta=1.09$ ; 95% CrI -0.27, 2.33; Bayesian  $P$ -value=0.06). We interpret this small estimated effect size, together with the fact that diet explained

a negligible proportion of the overall variance in body mass in this sample of individuals [marginal  $r^2=0.001$ , estimated using the 'r.squaredGLMM' function from the MuMIn package in R (<https://CRAN.R-project.org/package=MuMIn>)], as support for diet-independent body mass.

Knots exhibited large and significant changes in their activity budgets in response to experimental manipulations in diet (Table 3). Knots spent significantly more time feeding ( $\beta=3.21$ ; 95% CrI 2.98, 3.38) (Fig. 3B) and significantly less time active ( $\beta=-0.50$ ; 95% CrI -0.73, -0.40) (Fig. 3C) when provided with low-quality diets. Again, bivariate analyses indicate that there was scope for activity and feeding to be adjusted partially independently of one another. The estimated within-individual correlation was  $r=-0.32$  (95% CrI -0.44, -0.18). Finally, there was no support for an effect of diet quality on BMR ( $\beta=0.02$ ; 95% CrI -0.02, 0.06) (Table 3; Fig. 3D).

## DISCUSSION

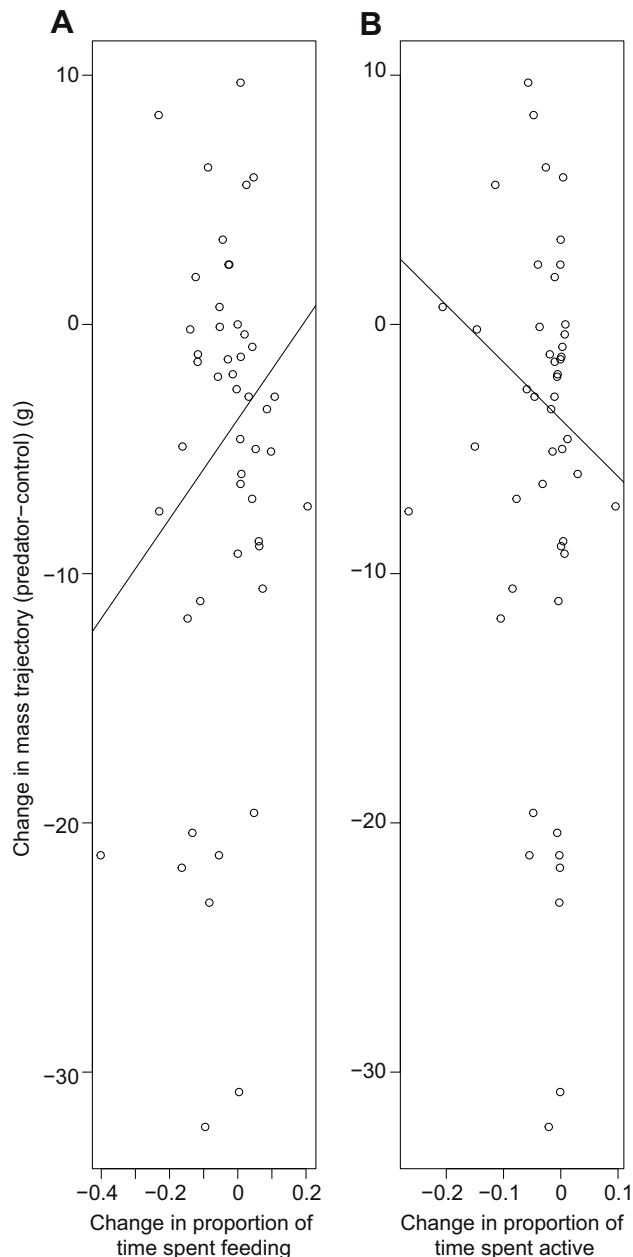
Here, we report the results of two studies that address the mechanisms of mass regulation in birds. We show that decreases in body mass in knots exposed to cues of predation coincide with decreased foraging effort and increased activity, and furthermore, that within-individual changes in mass trajectories can be predicted by individual differences in the extent of foraging restraint and activity up-regulation. We also provide evidence that both dieting and activity play a role in maintaining body mass under changing diet quality. When provided with low-quality diets, knots increased their time spent foraging, and decreased their time spent active. Although we found no support for diet-related differences in metabolic rate, we discuss the potential for metabolic adjustments to contribute to mass regulation in knots in view of our results (see below).

When exposed to model sparrowhawks over five consecutive days, red knot mass trajectories shifted significantly downwards. Reduction in body mass in response to increased perceived predation has been documented in several other studies (e.g. Abbey-Lee et al., 2016; Lilliendahl, 1997; MacLeod et al., 2005; Witter et al., 1994). However, such reductions are often assumed to come about because increased investment in vigilance or other anti-predator behaviours limits opportunities to search for food (Brown, 1999; Houston et al., 1993; Lima, 1998). While this may be the case in free-living birds, we show here that reductions in foraging effort also occur in captive birds with *ad libitum* access to food. We previously showed that the long-term up-regulation of vigilance in response to manipulated perceived predation danger meant that knots shifted the percentage of time spent vigilant from ~55% during the control treatment to 75% during the predator treatment

**Table 3. Experiment 2: sources of variation in foraging, activity and metabolic rate**

	Body mass (g)	ln (Time foraging)	ln (Time active)	Metabolic rate (W)
Fixed effects	$\beta$ (95% CrI)	$\beta$ (95% CrI)	$\beta$ (95% CrI)	$\beta$ (95% CrI)
Intercept*	132.29 (126.26, 137.78)	-4.08 (-4.19, -3.91)	-1.40 (-1.59, -1.26)	1.17 (0.87, 1.45)
Diet (low quality)	1.09 (-0.28, 2.32)	3.21 (2.98, 3.38)	-0.50 (-0.73, -0.40)	0.01 (-0.03, 0.06)
Random effects	$\sigma^2$ (95% CrI)	$\sigma^2$ (95% CrI)	$\sigma^2$ (95% CrI)	$\sigma^2$ (95% CrI)
Individual	188.02 (165.78, 214.55)	0 <sup>§</sup>	0.010 (0.006, 0.014)	0.021 (0.017, 0.028)
Aviary	4.83 (0.82, 14.40)	0.0030 (0.0006, 0.0077)	0.0015 (0.0004, 0.0044)	0.0005 (0.0001, 0.0019)
Treatment block	24.19 (18.61, 31.22)	0 <sup>§</sup>	0.0015 (0.0004, 0.0044)	0 <sup>§</sup>
Respirometry Chamber	n.a.	n.a.	n.a.	0.061 (0.047, 0.073)
Year	0 <sup>§</sup>	n.a.	n.a.	0.013 (0.006, 0.020)
Residual	49.99 (44.90, 57.45)	0.45 (0.35, 0.53)	0.35 (0.29, 0.44)	0.062 (0.05, 0.068)
Repeatability <sup>‡</sup>	$r$ (95% CrI)	$r$ (95% CrI)	$r$ (95% CrI)	$r$ (95% CrI)
Individual	0.79 (0.76, 0.82)	0 <sup>§</sup>	0.025 (0.018, 0.037)	0.27 (0.22, 0.32)

\*Intercept estimates are for high-quality diet. Diet estimate is the change in response variable for knots on low-quality diet relative to the high-quality diet. <sup>‡</sup>Adjusted repeatability calculated after correcting for fixed effects. <sup>§</sup>Variance components non-estimable. n.a., not applicable.



**Fig. 2. Within-individual changes in proportion of time spent feeding or active predict within-individual changes in mass trajectory in response to manipulations of perceived predation danger in red knots.** Results from Experiment 1. Each point represents an individual ( $N=64$  total). The lines fitted to the data are derived from the intercept and slope estimates from the mixed-effects model presented in Table 1.

(Mathot et al., 2009). Given that the mean proportion of time spent feeding and active during the control was  $<10\%$  each, neither of these necessarily had to be down-regulated to accommodate the increased vigilance. For example, time allocated to other behaviour (e.g. resting, roosting), could have decreased instead. We suggest that the predation-related decrease in mass trajectory appears to be brought about in part via reduced intake (i.e. dieting), and appears similar to the restraint in eating shown in a captive knot undergoing seasonal mass loss even while provided with *ad libitum* access to food (Piersma and Poot, 1993).

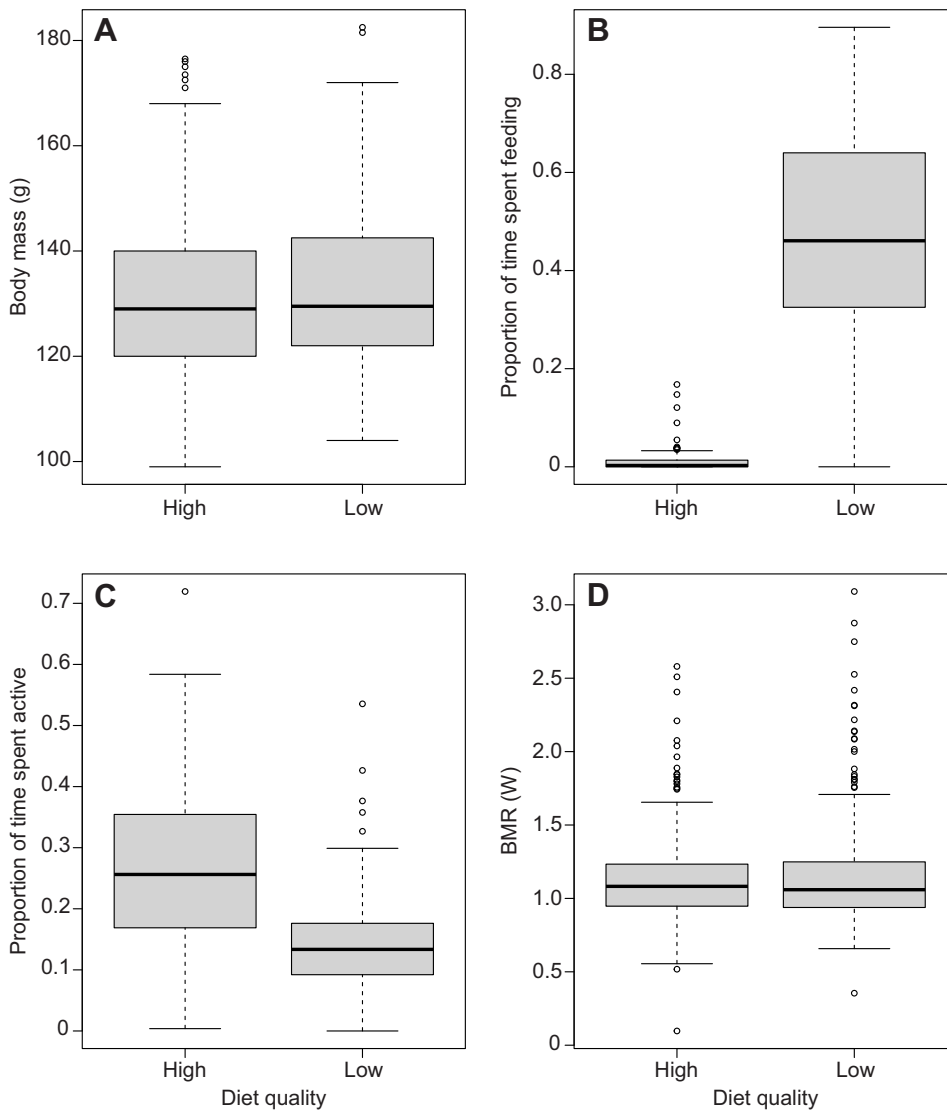
Importantly, time allocation trade-offs did not force a negative correlation between time spent active and time spent foraging. In red

knots, walking and probing the substrate increases energy expenditure by approximately 35% compared with resting (Piersma et al., 2003a). As such, we predicted that up-regulation in activity in response to the predator treatment might also contribute to mass loss. In contrast, we found that at the population level, activity decreased significantly during the predator treatment, although the magnitude of the decrease was small (Fig. 1C). This small decrease in percentage of time spent active was coincident with a large decrease in the percentage of time spent foraging. *Post hoc* analyses corroborate this result; at the within-individual level, there was a positive correlation between proportion of time spent active and proportion of time spent feeding ( $r=0.48$ ; 95% CrI 0.27, 0.66). Importantly, however, when considering within-individual changes in mass trajectories, time spent foraging and time spent active, there was strong support that both restraint in foraging and increased activity contributed to mass regulation (Fig. 2). Knots that showed the greatest decrease in the proportion of time spent foraging between predator and control treatments also showed the greatest decrease in mass trajectory. At the same time, knots that showed the greatest increase in time spent active between these treatments showed the greatest decrease in mass trajectories. Interestingly, these two mechanisms had very comparable effects on mass trajectories in terms of the magnitude of change in mass trajectory brought about per unit change in effort spent foraging or active (Table 2), suggesting that they are equally effective means of managing body mass in knots.

Our within-individual analyses also revealed moderate support for the interpretation that there was a downward shift in mass trajectory in knots between the predator and control treatment, even when there was no net change in foraging or activity (Table 2; intercept estimate). This could be due to the additional effects of the acute, short-term responses to predator exposure during which time steeper reductions in food intake occurred (i.e. indicating that the long-term response was an under-estimate of the overall average effect of predator treatment on knots) (Mathot et al., 2009). It may also indicate that additional, unmeasured mechanisms also contributed to the shift in mass trajectory, such as changes in digestive efficiency, or changes in metabolism (Halsey, 2018; Zanette et al., 2014). Although digestive efficiency is often stable across different food intakes and food qualities (Karasov, 1996; McWilliams and Karasov, 1998), we are not aware of any study investigating digestive efficiency as a function of predation risk. However, many birds defecate immediately prior to anti-predator escape flights (van der Veen and Sivars, 2000). While this may result in an immediate, small decrease in body mass (van der Veen and Sivars, 2000), it also implies that birds can plastically adjust gut transit time. All else being equal, decreasing gut transit time would provide a means of reducing the amount of energy extracted per unit of food consumed (i.e. reduce digestive efficiency; see Levey and Karasov, 1992; McWilliams and Karasov, 1998). Similarly, faecal energy loss has recently been proposed as an important mechanism contributing to energy homeostasis in humans (Lund et al., 2020). However, the role of changes in gut transit time as a mechanism of mass management in birds requires investigation.

Predator-related decreases in mass trajectories may also have resulted from metabolic adjustment. Indeed, when exposed to predators over short time scales, many organisms have been shown to exhibit increases in metabolic rate (Beckerman et al., 2007; Okuyama, 2015; Steiner and Van Buskirk, 2009). Although this is often interpreted as an unavoidable consequence of predator-induced stress, up-regulation of metabolic rates in response to increased perceived predation danger may also facilitate adaptive





**Fig. 3. The effect of high or low quality diet treatment in red knots.** Results from Experiment 2. The effect of diet quality on (A) body mass (g), (B) proportion of time spent feeding, (C) proportion of time spent active, and (D) basal metabolic rate (BMR, in W). Boxplots illustrate median (center line), upper and lower quartiles (upper and lower edges of box) and 1.5x interquartile range (whiskers). Sample sizes for each analysis were as follows: body mass ( $N=80$ ), proportion of time spent feeding ( $N=59$ ), proportion of time spent active ( $N=59$ ), BMR ( $N=80$ ). The same individuals are included in each diet treatment.

decreases in body mass over intermediate time scales. Studies tracking changes in metabolic rate across different time scales are needed to address this possibility.

In a second experiment, we manipulated diet quality provided to knots. We previously showed that large changes in the quality of the diets had no appreciable effect on overall body mass (Mathot et al., 2019). In the subset of birds included in the present study ( $N=59$ ), we again found that the diet manipulations used here had no biologically important effect on body mass when birds were given sufficient time to acclimate to the diet. The maintenance of body mass under decreasing energetic quality of food was achieved through significant up-regulation in foraging effort (from ~1% of time spent foraging to ~45% of time spent foraging), and a concomitant decrease in the proportion of time spent active (from ~25% to 15%). Several studies have shown that the increased foraging intake necessitated by low-quality diets results in substantive increases in the gizzard (i.e. muscular stomach used for food processing) (e.g. Bijleveld et al., 2014; Dekinga et al., 2001; Mathot et al., 2017). This was also true in this cohort of birds; changes in diet quality were associated with a twofold difference in gizzard mass (Kok et al., 2019; Mathot et al., 2019). Importantly, the magnitude of the shift in the percentage of time spent foraging observed in this study (from 1.5 to 47.5%) was not sufficient to

force a coincident change in activity. The percentage of time active during the high-quality diet treatment (27%) could have been maintained on the low-quality diet while still allowing nearly 25% of the overall activity budget for other behaviours such as preening or roosting. *Post hoc* analyses corroborate this interpretation. Although there was a negative within-individual correlation between proportion of time spent active and proportion of time spent feeding ( $r=-0.32$ ; 95% CrI  $-0.44, -0.18$ ), the correlation was moderate. This suggests that there was at least partial independence in how time was allocated to these two categories of behaviour, as complete dependence would have resulted in a correlation of  $-1$ . Taken together, we suggest that the changes in activity patterns may have been adopted as a means of conserving energy under conditions of low diet quality.

We also investigated whether diet-induced changes in metabolic rate could have contributed to the observed mass regulation. All else being equal, lower BMR under conditions of low food quality would facilitate mass regulation. We found no support for diet-induced changes in BMR. However, changes in diet are known to induce changes in body composition, which may complicate interpretation of this null result. For example, in knots, low-quality diets result in increases in gizzard mass (Bijleveld et al., 2014; Dekinga et al., 2001; Mathot et al., 2019), and decreases in pectoral



muscle mass (Mathot et al., 2019). These muscles each have high metabolic activity, although gizzard is more metabolically active compared with pectoral muscle (i.e. contributes more to basal metabolic rate per gram of tissue) (Daan et al., 1990; Kersten and Piersma, 1987; Piersma et al., 1996). In a previous analysis with the same set of birds, we showed that diet-induced changes in the mass of gizzard and pectoral muscle were roughly equal, but in opposite directions; for each 1 g increase in gizzard mass within individuals, there was on average a 1.26 g decrease in pectoral muscle mass (Mathot et al., 2019). Thus, any diet-induced changes in BMR due to increased gizzard mass may have been offset by the concomitant changes in pectoral muscle mass.

In contrast with our results, an earlier study found that knots switched from Trouvit (the same high-quality food as used in the current study) to blue mussels (*Mytilus edulis*) exhibited increases in total body mass, gizzard mass and decreases in BMR after 3 weeks on the low-quality diet (Piersma et al., 2004). However, there are key differences between our study and the study by Piersma et al. (2004) that may account for the contrasting results. The knots used in the current study were recently wild-caught, and repeatedly shifted between high and low digestive quality diets. In contrast, the knots used in the study by Piersma et al. (2004) had been in captivity for 5–6 years prior to the experiments and had been maintained on *ad libitum* Trouvit since capture. Given that knots generally exhibit a reluctance to switch from high-quality to low-quality food (Piersma et al., 1993), in the earlier study by Piersma et al. (2004) 3 weeks on a diet of blue mussels may not have been sufficiently long for knots to fully acclimate to the change in diet. Studies tracking changes in gizzard mass and metabolic rate in response to diet quality manipulations over different time scales would help clarify whether metabolic responses to diet quality (in knots and other birds) are indeed time dependent.

Taken together, these two experiments provide evidence that adjustments in foraging effort and activity in red knots contribute to patterns of mass regulation. Foraging decreased under increased predation danger, which facilitated mass loss relative to the low danger treatment. Foraging also decreased when knots were provided higher quality food. However, the absolute investment in foraging on the low-quality diet was markedly different across the two experiments; proportion of time spent foraging was 0.10 in experiment 1 versus 0.40 in experiment 2 (see Figs 1 and 3). Although some of this might reflect variation in the quality of *Peringia* provided, differences in the housing densities of knots across the two experiments (experiment 1: 0.16 knots m<sup>-2</sup>; experiment 2: 1.75 knots m<sup>-2</sup>) probably also resulted in differences in perceived competition and the degree of interference competition, which has previously been shown to result in increased time spent searching in a food patch (Bijleveld et al., 2012).

Interestingly, voluntary flight did not appear to play an important role in mass regulation in either experiment. In the first experiment, no voluntary flight was observed, and in the second experiment, voluntary flight made up a very small percentage of the total activity budgets (<1%). Voluntary flight can result in energy expenditure of nine to ten times basal metabolic rate (Piersma and van Gils, 2011), and would therefore be a very effective means of increasing energy expenditure. Our failure to detect voluntary flight as a mechanism of mass management may be because our focal observations were too short to capture this relatively rare behaviour, or because voluntary flight is not used to manage body mass in captive knots. The latter might be the case because the experimental arena and aviaries used in the present study are too small to allow for unencumbered flight. Alternatively, flight may impose additional, non-energetic costs.

For example, voluntary flight for mass loss may increase an individual's vulnerability to predation by reducing flock cohesion (Mathot et al., 2009). Ultimately, longer observation sessions are needed to assess the potential for small shifts in voluntary flight as a mechanism of body mass management.

## Conclusion

We show that both adaptive changes in body mass, and maintenance of body mass under changing conditions, coincide with changes in foraging effort and activity. Loss of body mass is often seen as a consequence of missed foraging opportunities when mitigating predation danger (e.g. due to increase of vigilance, or avoiding risky activities). However, our results suggest that birds are able to exhibit restraint in foraging, analogous to 'dieting'. Under conditions of *ad libitum* access to food, knots spent less time feeding when weight reduction was adaptive (experiment 1), and when the food provided was of higher energetic quality (experiment 2). Although the second experiment provides no evidence that mass regulation was achieved through metabolic adjustment, the regulation of body mass was studied over a long time period, once body mass had the opportunity to stabilize (i.e. >3 weeks adjustment to the new diet). Thus, we cannot rule out the possibility that metabolic adjustments contributed to mass regulation over shorter time scales (e.g. the first few days adjusting to a new diet). Indeed, experiment 1 provides some evidence that additional mechanisms, such as metabolic adjustment, could have contributed to mass regulation, and other studies have also suggested that metabolic adjustments have time-dependent effects. Understanding whether, and under what circumstances, metabolic adjustments play a role in mass regulation in birds requires further investigation.

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## Competing interests

The authors declare no competing or financial interests.

## Author contributions

Conceptualization: K.J.M., E.M.A.K., P.v.d.H., A.D., T.P.; Methodology: K.J.M., E.M.A.K., P.v.d.H., A.D., T.P.; Formal analysis: K.J.M.; Investigation: K.J.M., E.M.A.K., P.v.d.H., A.D.; Resources: T.P.; Data curation: K.J.M., E.M.A.K., P.v.d.H.; Writing - original draft: K.J.M.; Writing - review & editing: E.M.A.K., P.v.d.H., A.D., T.P.; Visualization: K.J.M.; Supervision: K.J.M., E.M.A.K., P.v.d.H., T.P.; Project administration: K.J.M., E.M.A.K., P.v.d.H.; Funding acquisition: K.J.M., T.P.

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## Data availability

All data and R scripts required to reproduce the analyses presented in this manuscript are available from the Open Science Framework digital repository (<https://osf.io/uswk7/>).

## Supplementary information

Supplementary information available online at  
<https://jeb.biologists.org/lookup/doi/10.1242/jeb.231993.supplemental>

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## Electronic supplementary material

**Table S1:** Ethogram used for behavioural observations in Experiment 2. Type refers to whether the behaviour was scored as a state or point event. State events are scored for their whole duration; any given state event continues until a new state event is registered. Point events are scored for their number of occurrences. These were generally behaviours that were too short in duration to allow for accurate duration estimates. Activity was estimated as the sum of time spent walking, flying and searching (see main text).

Behaviour	Description	Type
Walking	Stepping forward, backward, or on one side in order to move to another location	State
Flying	Taking off by flapping, and move to another location	State
Resting	Standing still without any visible action	State
Roosting	Standing with its bill between the feathers, often on one leg	State
Vigilance	Looking around with a horizontal bill and/or looking up by tilting the head	State
Preening	Cleaning or rearranging its feathers with the bill or by flapping, scratching with one of its feet	State
Stretching	Stretching wings and/or legs	Point
Ruffle	Shaking all its feathers	Point
Eating	Pecking some food of the feeder and swallowing it	State
Drinking	Putting its bill in the water, then turning its head up to send the water to the back of his throat	State
Pecking	Pecking solid objects with the bill (often repeatedly)	State
Searching	Looking at the ground in order to find food with the bill forming a 45° angle with the horizontal, or probing in the sand	State
Pooping	The bird releases excrement from the body	Point
Bathing	Cleaning its feathers using water, often shaking the body	State
Vocalizing	Producing vocalisations	Point
Interaction initiator	The bird attacks one of the other bird	Point
Interaction victim	The bird is being attacked by one of the other birds	Point
Miscellaneous	Behaviour that does not fit the descriptions above	Point
Out of sight	The bird cannot be seen by the observer	State



**Figure S1.** Schematic representation of an experimental block in experiment 1. Black rectangles represent each day of the block (1 through 5). On each day, two predator events occurred (purple = presentation of a perching sparrowhawk, green = presentation of a gliding sparrowhawk), with behavioural observations conducted immediately before and after each presentation (small grey and yellow rectangles). Only the observation periods shown in yellow were used to calculate long-term behavioural responses, as they reflected carry-over responses from presentations on the previous day. Mass of all individuals was calculated at the beginning and end of each block. For the control (i.e., non-predator) block of the experiment, the same sampling design was used except that no predators were presented (blank controls).

