

## Chapter 5

# Diet preferences as the cause of individual differences rather than the consequence

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**ABSTRACT**

Behavioural variation within a species is usually explained as the *consequence* of individual variation in physiology. However, new evidence suggests that the arrow of causality may well be in the reverse direction: behaviours such as diet preferences cause differences in physiological and morphological traits.

Recently, diet preferences were proposed to underlie consistent differences in digestive organ mass and movement patterns (patch residence times) in red knots (*Calidris canutus islandica*). Red knots are molluscivorous and migrant shorebirds for which the size of the muscular stomach (gizzard) is critical for the food-processing rate. In this study, red knots (*C. c. canutus*,  $n = 46$ ) were caught at Banc d'Arguin, an intertidal flat ecosystem in Mauritania, and released with radio-tags after the measurement of gizzard mass. Using a novel tracking system (time-of-arrival), patch residence times were measured over a period of three weeks. Whether or not gizzard mass determined patch residence times was tested experimentally by offering 12 of the 46 tagged red knots soft diets prior to release; this reduced an individual's gizzard mass by 20–60%. To validate whether the observed range of patch residence times would be expected from individual diet preferences, we simulated patch residence times as a function of diet preferences via a simple departure rule.

Consistent with previous empirical studies, patch residence times in the field were positively correlated with gizzard mass. The slope of this correlation, as well as the observed range of patch residence times, were in accordance with the simulated values. The 12 birds with reduced gizzard masses did not decrease patch residence times in response to the reduction in gizzard mass. These findings suggest that diet preferences can indeed *cause* the observed among-individual variation in gizzard mass and patch residence times. We discuss how early diet experiences can have cascading effects on the individual expression of both behavioural and physiomorphic traits. This emphasises that to understand the ecological consequences of individual differences, knowledge of the environment during development is required.

## INTRODUCTION

Individuals are often constrained in the expression of behavioural traits relative to the overall range of expression in the population (Réale & Dingemanse 2010). Individual-specific behavioural characteristics have been captured with the terms 'behavioural syndromes' and 'animal personality' (Sih, Bell & Johnson 2004; Réale *et al.* 2010). Behaviour has often been regarded as flexible, with behavioural differences being considered to result from individual-specific physiological and morphological characteristics (Krebs & Davies 1997). Many morphological and physiological traits are highly plastic too (Pigliucci 2001), even into the adult stage (Piersma & van Gils 2011). Consequently, the causal direction of a correlation between what we will subsequently call 'physiomorphic' traits and behavioural traits is not self-evident (see also Stamps 2003). The two can be seen as complementary aspects of the phenotype (Dingemanse *et al.* 2010), both of which will be shaped during ontogeny in interaction with each other and the environment (Stamps 2003). Hence, behavioural syndromes may also *cause* consistent variation in others traits, be it behavioural or physiomorphic (e.g. Eklöv & Svanbäck 2006; Bijleveld *et al.* 2014, 2016).

Individual diet preferences are among the best studied behaviours, and consistent differences therein have been shown to result from dietary experiences early in life (Burghardt & Hess 1966; Provenza & Balph 1987; Estes *et al.* 2003; Villalba, Provenza & Han 2004; Darmaillacq, Chichery & Dickel 2006). Hence, the early development of diet preferences may well function as the basis of individual variation in other traits later in life. Variable dietary experiences are more likely when the availability of different food sources is variable. This is the case for red knots (*Calidris canutus*, Linnaeus), a medium-sized migrating shorebird (Piersma 2007; Buehler & Piersma 2008) that primarily feeds on molluscs (Prater 1972; van Gils *et al.* 2005a). The quality and diversity of the food landscape that they live in is variable in space (Compton *et al.* 2013) as well as in time (Kraan *et al.* 2013; Chapter 3). In captive as well as free-living red knots (*C. c. islandica*), diet preferences were put forward as a possible cause of differences in movement behaviour and digestive organ mass (Bijleveld *et al.* 2014; Bijleveld *et al.* 2016). Red knots ingest their mollusc prey whole and crush them in the gizzard (Piersma, Koolhaas & Dekinga 1993), the size of which can be measured non-invasively by ultrasonography (Dietz *et al.* 1999). Gizzard mass was shown to be highly variable between individuals and flexible within individuals and to reflect the digestive quality of the previous diet (where prey quality is measured as ash-free dry flesh mass divided by dry shell mass; Piersma, Koolhaas & Dekinga 1993; Dekinga *et al.* 2001; van Gils *et al.* 2003a; Chapter 4).

In an experiment with captive red knots, gizzard mass was positively correlated with the average duration of patch visits (patch residence time) (Bijleveld *et al.* 2014). The lack of behavioural change after manipulating an individual's gizzard mass suggested that variation in digestive organ mass resulted from the consistent behavioural differences, rather than the other way around. Measurements in the wild also showed that gizzard mass was negatively correlated with the average digestive quality of prey in their selected habitat (van Gils *et al.* 2005b; Bijleveld *et al.* 2016). Together, these findings suggest that

the individual variation in gizzard mass and differences in movement behaviour may be consequences of variation in diet preferences between individuals, and ask for an experimental test under natural conditions.

This study provides: (i) field measurements of the correlation between patch residence times and gizzard masses; (ii) an experimental test of the causal direction of this correlation; and (iii) a conceptual mechanism to explain the observed differences in patch residence times between red knots as a function of diet preferences. Using the novel time-of-arrival tracking system (MacCurdy, Gabrielson & Cortopassi 2012; Piersma *et al.* 2014; Bijleveld *et al.* 2016), patch residence times were measured in 34 free-living red knots (*C. c. canutus*) on the intertidal flats of Banc d'Arguin, Mauritania. Additionally, after having been held captive for two weeks on diets of medium (six birds) and high digestive quality (six birds), 12 tagged red knots were released with reduced gizzard masses. To test whether this manipulation caused a decline in patch residence times after release in the wild, we compared the observed relation between gizzard mass and mean patch residence time for these 12 treated birds (to be referred to as 'treatment birds') with the 34 unmanipulated birds (to be referred to as 'reference birds'). Finally, the observed range of patch residence times and gizzard masses was compared to simulated patch residence times where animals were assumed to have constant patch giving-up times, but differ in the minimum digestive quality of accepted prey.

## MATERIALS AND METHODS

### Time-of-arrival tracking

Between 9 January and 13 February 2013, 46 red knots were tracked with the time-of-arrival tracking system (MacCurdy, Gabrielson & Cortopassi 2012; Piersma *et al.* 2014; Bijleveld *et al.* 2016) in the Baie d'Aouatif in Parc National du Banc d'Arguin, Mauritania, West Africa (19°53'N, 16°17'W) (Piersma *et al.* 2014). The birds were caught adjacent to the islet Zira, on the southwest entrance of the bay, using mist nets (14 birds, between 8 and 11 January) and a cannon net (32 birds, 12 January). All birds were released with a 6-g (range 5.5 – 6.5 g) tag glued to the skin of their rump with cyanoacrylate (Warnock & Warnock 1993). Gizzard mass was measured by ultrasonography (Dekinga *et al.* 2001; Chapter 4) within 4 h after catch. The 34 reference birds were released within 1 day after catch between 9 and 12 January (Fig. 5.1), except for one bird that showed signs of illness after being caught on 12 January. It was released in a healthy condition and with a tag on 20 January and was omitted from the analyses. The 12 treatment birds were released on 23 January after 11 days of captivity.

Each tag emitted a tag-specific radio signal each second, which could be received by up to nine radio receiver stations placed in the area (Piersma *et al.* 2014, Fig. A5.1). These stations then registered the time-of-arrival of the tag-specific signal. The differences in signal arrival times between the stations were used to calculate the tag's position (MacCurdy, Gabrielson & Cortopassi 2012). Position error estimates were produced when the signal was received by at least four stations (MacCurdy, Gabrielson & Cortopassi

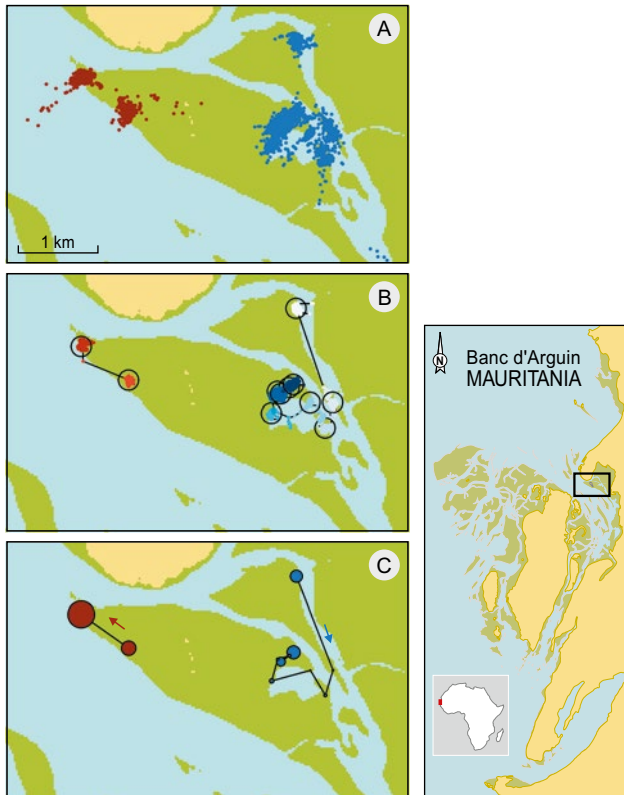


**Figure 5.1** Christine Lagarde, director of the International Monetary Fund, visits Parc National du Banc d'Arguin, Mauritania, on 10 January 2013. Here she has just personally released one of the radio-tagged red knots at the shore of the fishing village Iwik. Banc d'Arguin is the most important wintering site for Palearctic shorebirds, and comprises the majority of Mauritania's coast line. It is declared by UNESCO as a site of outstanding universal value. Nonetheless, this pristine state is threatened by international offshore fisheries, urban development, and an increase of unregulated fisheries inside the national park. Photo credit: Marieke Feis.

2012). Signals that were received by less than four stations were not considered for the analyses, and only position estimates with an error above 125 m (the radius of residence patches). For comparing the treatment group with the reference group, only data from 23 January onwards were used for the analyses, which is the date when the treatment birds were released.

### Summarizing the tracking data into patch residence times

To calculate the mean patch residence times of each bird during each low tide, the position estimates were combined into residence patches (Bijleveld *et al.* 2016) according to the method of Barraquand & Benhamou (2008). We will describe the basics only and refer to both papers for detailed methodology. First, the data was median-filtered with a 5-points sliding window to reduce the error. For each position estimate (Fig 5.2A), the duration of the stay within 125 m of that position, without any excursions outside the radius



**Figure 5.2 Example of time-of-arrival tracking data.** (A) The dots show the estimated positions of two randomly chosen red knots during one low tide in the Baie d'Aouatif. The underlying map shows the mudflats that are exposed during low tide. (B) The position estimates are combined into residence time patches, shown by open circles. The median-filtered data underlying patches are shown in different shades. Subsequent positions are connected by lines. (C) Circles show the sequence of patch visits in the direction of the arrows. The size of circles show the relative duration of patch visits, that is patch residence times.

for more than 30 s, was calculated. Subsequently, the sequence of residence times was segmented into so-called residence patches, using the penalized contrasts method described by Lavielle (2005). Then, adjacent residence patches of which the median position was within 125 m of each other were combined (Fig. 5.2B). Finally, patch residence times were calculated as the interval between the first and last position estimate within a patch (Fig. 5.2C).

Because we were interested in low-tide (foraging) distributions, patch visits that started or ended within 2 h before and after astronomical low tide (4.5 h after Dakar, retrieved from tides.mobilegeographics.com) were selected. Patch visits that extended into the high tide (2 h before to 2 h after astronomical high tide) were removed from the analysis (80 of 3141 patches), as it was likely that birds were roosting. Patches visited shorter than 10 min were not considered ( $n = 167$ ), as they might indicate bouts during which birds were travelling (Bijleveld *et al.* 2016). Finally, when not receiving signals from a tag for more than 1 h, this bird during that low tide was excluded from the analysis (98 of 1019 cases). Patch residence times were log-transformed and then averaged per bird per tide. This resulted in a total of 921 mean patch residence times from 35 birds (26.3 per bird, SD = 11.5), of which 337 were from the 12 treatment birds (28.1 per bird, SD = 11.2).

### Experimental treatment

Twelve red knots, caught by cannon net on 12 January, were kept for eleven days in two in-house aviaries ( $1.5 \times 1 \times 0.5$  m) at the scientific station adjacent to the Baie d'Aouatif. The birds were divided at random into two treatment groups of six birds each. To reduce gizzard mass of individuals, one group received only the flesh of the bivalve *Senilia senilis* (see also Chapter 4). In an attempt to maintain original gizzard masses, the other group was additionally offered 1200 hard-shelled *Dosinia isocardia* per day. These prey were collected every day in the Baie d'Aouatif. All birds had ad libitum access to fresh water. Gizzard masses were measured within four hours after catch and on the evening before the release. Gizzard mass of the birds on the soft diet decreased from  $8.7 \pm 0.5$  g at the day of catch to  $5.0 \pm 0.5$  g on the day before release (mean  $\pm$  SE,  $t = -5.6$ ,  $P = 0.0002$ ). Gizzard mass of the birds on the partly hard-shelled diet decreased from  $9.2 \text{ g} \pm 0.7$  at catch to  $6.6 \text{ g} \pm 0.7$  before release ( $t = -2.8$ ,  $P = 0.02$ ); the decrease in gizzard mass did not differ significantly between treatment groups ( $t = 1.4$ ,  $P = 0.20$ ), and the gizzard masses were not significantly different at release either ( $t = 1.9$ ,  $P = 0.07$ ).

### Prey density, prey quality and giving-up times

The study area was systematically searched for tagged red knots. On 44 occasions, tagged birds were filmed from a distance of  $\sim 200$  m. Excluding cases in which birds were obviously disturbed by the observers, 12 giving-up times were measured, where giving-up time is defined as the time between last prey encounter and take-off. At each of the 44 locations, next to where the bird left traces (footprints, droppings or probing holes), eight sites were marked with small pegs and sampled for prey availability within the following week. Each sample was taken with a PVC-core (diameter 15 cm) to a depth of 20 cm in the sediment.

The top 4 cm (coinciding with the maximum probing depth of red knots) was separated and sieved over a 1-mm mesh. All molluscs were sorted in the field station and brought to NIOZ (the Netherlands) in a 4% formaldehyde solution, where they were identified to genus level and measured to the nearest 0.1 mm. Mollusc density was calculated by dividing the summed number of observed molluscs by the summed sampled surface per location ( $0.14 \text{ m}^2$ ,  $n = 8$ ). Only prey of ingestible sizes were considered (Zwarts & Blomert 1992).

To determine dry flesh mass and dry shell mass ( $DM_{\text{shell}}$ ), flesh and shell were separated for a subset of all bivalves and the gastropod *Bulla* sp., dried at  $60^\circ\text{C}$  for 3 days and weighed (mg). Flesh was then incinerated for 5 h at  $560^\circ\text{C}$  and weighed again to determine ash-free dry flesh mass ( $AFDM_{\text{flesh}}$ ). Flesh and shell could not be separated in gastropods other than *Bulla*. These gastropods were incinerated whole, assuming that 12.5% of organic matter resides in the shell (Dekker 1979). By linear regression on log-transformed values,  $AFDM_{\text{flesh}}$  and  $DM_{\text{shell}}$  were estimated as a function of shell length for each species separately. Based on these regressions, digestive quality was estimated for each individual prey by dividing  $AFDM_{\text{flesh}}$  by  $DM_{\text{shell}}$  (van Gils *et al.* 2005b). To arrive at the estimated prey quality distribution to be used in the simulations, the observed variation in digestive quality was taken into account by adding to the estimates a value drawn randomly from the normal distribution (Gaussian noise), with the standard deviation as measured for the concerning prey species.

## Statistics

First, the slope of the correlation between gizzard mass and patch residence times was tested in the reference birds. Secondly, we determined whether the treatment birds adjusted their patch residence times as a consequence of the change in gizzard mass. We did this by testing whether the treatment birds obeyed the observed relationship given their gizzard mass at release and whether there was a difference in the response of the two treatment groups. Thirdly, we tested whether the treatment birds obeyed the observed correlation given their gizzard mass at catch, which would alternatively suggest that the treatment birds did not adjust patch residence times to their manipulated gizzard mass.

The correlation between patch residence time and gizzard mass in the reference birds was tested by comparing linear mixed effects models (function 'lmer' in R package 'lme4'; Bates *et al.* 2015; R Core Team 2015), including low-tide ID and bird ID as random effects. Patch residence times and gizzard mass were log-transformed before the analysis. In addition to gizzard mass, time-of-day and low-tide water level were included as explanatory variables. When astronomical low tide was within 2 h before sunrise and 2 h after sunset, the low tide period was classified as 'day', and otherwise as 'night'. All possible combinations of variables were compared, including all interactions between two variables, but not more than two, resulting in 14 different candidate models. Model selection was performed by calculating AICc-weights of all the candidate models (Burnham & Anderson 2002). Models were regarded as competitive to the best model (the model with lowest AICc-score) if the cumulative AICc-weight was below 0.95 and did not contain uninformative parameters (parameters that did not decrease AICc-score when added to the model; Arnold 2010).



Because the duration of each low tide is fixed, mean patch residence time per tide was negatively collinear with the number of patches visited per tide and did not follow a normal distribution, nor any other parametric distribution. However, a histogram of the model residuals and a plot of the residual values against the fitted values did not show strong violation of normality assumptions. An alternative analysis was performed on the number of patches visited per tide instead of mean patch residence time. Although this alternative procedure gave the same results and conclusions (not shown), this method was not preferred because 234 out of 584 data points had to be removed, since the number of patch visits could not be accurately assessed when birds were out of range of the receiver stations during parts of the low tide period.

Because patch residence times could not be measured in the treatment birds before they underwent the treatment, we assumed that the relation between patch residence times and gizzard mass before treatment was the same as in the reference birds. Hence, if the treatment had no effect, patch residence times after the treatment should have the same relation with gizzard mass *at catch* as found for the reference birds. On the other hand, if the treatment birds adjusted patch residence times to their gizzard mass after the treatment, the relation between gizzard mass *at release* and patch residence times should be the same as observed in the reference birds. This was tested by comparing the explanatory power of linear mixed-effects models with and without including a treatment parameter, fitted on the data of both the reference birds and the treatment birds, using either gizzard mass *at catch* or gizzard mass *at release*. The coefficients for the model intercept and log-transformed gizzard mass were constrained to the values that were estimated for the reference birds alone (by specifying an 'offset' in the function 'lmer'). Additional models were added to test for an effect of diet on patch residence times within the treatment birds, even though no effect of diet group on gizzard mass was found. To reveal a potential treatment effect that wore off after a few days in the field, which may be masked in the analysis of the full 3 weeks after release of the treatment birds, an additional analysis was performed with only the data of the first 2 days after the day of release.

### **A simulation to explain differences in patch residence times from diet preferences**

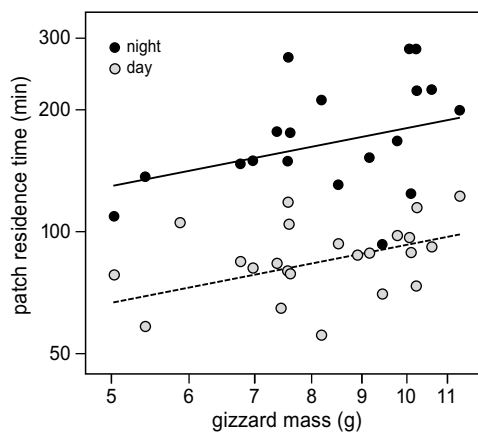
Foragers may individually differ in the minimum prey quality that they accept. Given that the decision of a foraging animal to leave a patch should be related to the encounter rate of acceptable prey at that patch, this is likely to affect average patch residence time (Charnov 1976). Red knots foraging on mollusc prey were shown to increase acceptance probability for prey of higher quality (van Gils *et al.* 2005b). Diet quality was expressed in terms of digestibility, measured as the amount of ash-free flesh mass per unit of dry shell mass. In red knots, gizzard masses were shown to reflect the digestive quality of the previous diet (Dekinga *et al.* 2001). Hence, if birds maintain the minimum gizzard mass that is needed to fulfil energy demands on prey of minimum acceptable digestive quality (at an ash-free dry flesh intake rate of 0.2 mg/s; van Gils *et al.* 2009), gizzard mass and patch residence time are expected to correlate. This expectation was formalized in a simulation.

We assumed a patchy distribution of food that varies in quality, and foragers that depart from a patch when prey of acceptable quality is not encountered within a fixed period of time (giving-up time; Krebs, Ryan & Charnov 1974). More sophisticated and perhaps more realistic behavioural rules exist (McNair 1982; Olsson & Holmgren 1998; van Gils *et al.* 2003b), but the predictions with the simple fixed giving-up time rule come reasonably close (with an approximately 10% lower encounter rate than when using more complex rules; Green 1984). Assuming that individuals do not differ in giving-up times, the predicted patch residence time is dependent on the total prey density and the prey quality distribution. Expected patch residence times were simulated for a sequence of minimum acceptable prey qualities by repeatedly drawing expected search times from an exponential distribution (Rita & Ranta 1998), where the average encounter rate ( $1/\text{search time}$ ) was defined as the average searching efficiency (de Fouw *et al.* 2016) times the density of acceptable prey. Patch residence time was then defined as the cumulative search time until search time exceeded the giving-up time, plus the cumulative handling time on all accepted prey. This procedure was repeated 100 times for each of the 44 locations where prey density and prey digestive quality were measured. A detailed description is given in Appendix A5.1.

## RESULTS

### Patch residence times of reference birds

As expected, patch residence times of the reference birds showed a positive loglinear correlation with gizzard mass (Fig. 5.3, model 1.1 in Table 5.1). Patch residence times were longer in the night than during the day, but their correlation with gizzard mass did



**Figure 5.3 Patch residence times of reference birds increase with gizzard mass.** Dots show the duration of patch visits per bird, averaged per low tide and then over all low tides. Regression lines show predicted values (model 1.1 in Table 5.1), which include the differences between low tides in the night (solid line) and during the day (dashed line). Note that the axes are log-scaled.

not differ between day and night (i.e. no interaction was observed between time-of-day and gizzard mass, Fig. 5.3, model 1.3 in Table 5.1). The low-water level did not explain any variation in patch residence times (model 1.2 in Table 5.1). Patch residence time estimations of the best model ranged from 67 min (95% CI: 54–82 min) for birds with a 5-g gizzard to 97 min (95% CI: 83–114 min) for birds with a 11-g gizzard during the day, and from 130 (95% CI: 96–174 min) to 189 min (95% CI: 146–244 min) during the night (Fig. 5.3, see table A5.1 for model 1.1 parameter estimates). The model without gizzard mass and only time-of-day showed very little support (model 1.7, AICc-weight = 0.05).

**Table 5.1 AICc comparison of statistical models.**

Model	Fixed effects <sup>a</sup>	K <sup>b</sup>	ΔAICc	AICc weight	Cum. weight	LL <sup>c</sup>
<b>1.1</b>	<b>Gizzard + daytime</b>	<b>6</b>	–	<b>0.43</b>	<b>0.43</b>	<b>–76.1</b>
1.2	Gizzard + daytime + height	7	2.01	0.16	0.59	–76.1
1.3	Gizzard × daytime	7	2.04	0.15	0.74	–76.1
1.4	Gizzard × height + daytime	8	3.49	0.07	0.81	–75.8
1.5	Gizzard × daytime + height	8	4.06	0.06	0.87	–76.1
1.6	Daytime × height + gizzard	8	4.06	0.06	0.93	–76.1
<b>1.7</b>	<b>Daytime</b>	<b>5</b>	<b>4.34</b>	<b>0.05</b>	<b>0.98</b>	<b>–79.3</b>
1.8	Daytime + height	6	6.37	0.02	0.99	–79.3
1.9	Daytime × height	7	8.42	0.01	1	–79.3
1.10	Gizzard + height	6	17.85	0	1	–85.0
1.11	Gizzard	5	18.34	0	1	–86.3
1.12	Gizzard × height	7	19.15	0	1	–84.7
1.13	Height	5	22.60	0	1	–88.4
1.14	1	4	22.89	0	1	–89.6
<b>2.1</b>	<b>Offset(model 1.1, giz at catch) + treatment</b>	<b>4</b>	–	<b>0.69</b>	<b>0.69</b>	<b>–121.6</b>
2.2	Offset(model 1.1, giz at catch) + treatment + diet	5	2.01	0.25	0.94	–121.6
<b>2.3</b>	<b>Offset(model 1.1, giz at catch)</b>	<b>3</b>	<b>4.76</b>	<b>0.06</b>	<b>1</b>	<b>–125.0</b>
<b>3.1</b>	<b>Offset(model 1.1, giz at release) + treatment</b>	<b>4</b>	–	<b>0.72</b>	<b>0.72</b>	<b>–122.4</b>
3.2	Offset(model 1.1, giz at release) + treatment + diet	5	1.93	0.28	1	–122.4
3.3	Offset(model 1.1, giz at release)	3	20.02	0	1	–133.4

NB. Linear mixed-effects models (function ‘lmer’ in R package ‘lme4’; Bates *et al.* 2015; R Core Team 2015) with tide ID and bird ID as random intercepts. Best models and competitive models are in boldface (Burnham & Anderson 2002).

Parameters were estimated by maximizing the log likelihood. Log-transformed patch residence time is the response variable in all models, which is averaged per bird per low tide after transformation.

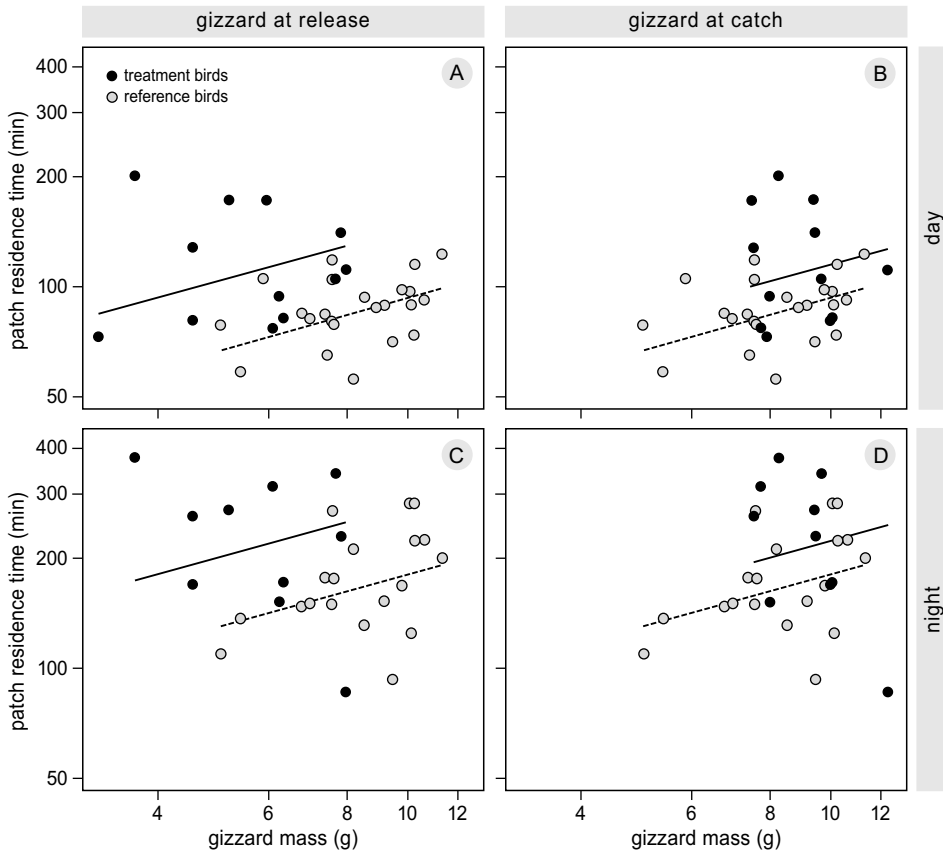
<sup>a</sup> In models 1, ‘Gizzard’ refers to gizzard mass at catch. ‘Daytime’ is a factor with two levels: day and night. ‘Height’ refers to the astronomical water level at the specific low tide. To test whether the treatment birds deviate from the predictions derived from the reference bird data, the estimated coefficients of the fixed effects of model 1.1 are used as an offset in models 2 and 3. Models 2 contain gizzard masses as measured when the birds were caught; models 3 contain gizzard masses before release. Factor ‘Treatment’ refers to whether the bird was in the treatment group or the reference group. ‘Diet’ refers to the diet group within the treatment group (either a soft diet or a partially hard-shelled diet).

<sup>b</sup> The number of parameters in the model.

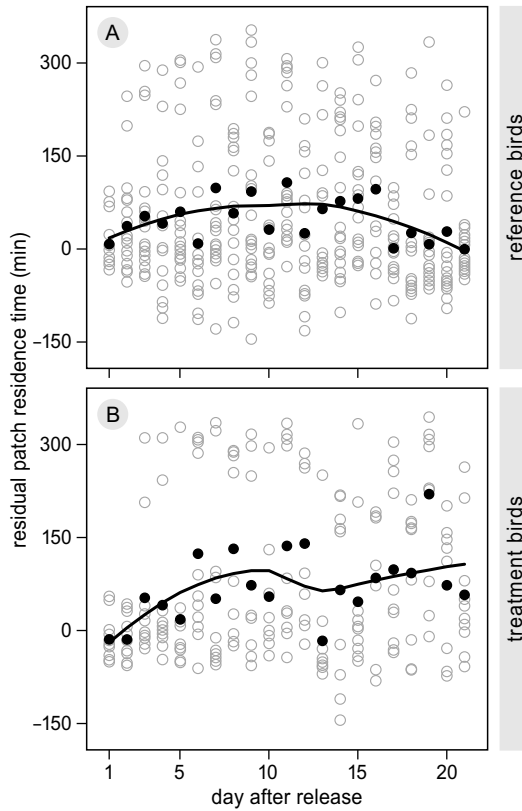
<sup>c</sup> Log-likelihood.

### Patch residence times of treatment birds

The treatment birds had longer residence times than predicted on the basis of their gizzard mass at release (on average 58 min, Fig. 5.4A and C, model 3.1 in Table 5.1), and no effect of treatment group was observed. This suggests that the birds did not adjust patch residence time to their new gizzard mass. Patch residence times was also longer than expected from gizzard mass at catch (on average 31 min, see Fig. 5.4B and D, model 2.1 in Table 5.1). With an AICc-weight of 0.06, the model without a treatment effect had little support (model 2.3 in Table 5.1). No difference was found between the diet groups within the treatment birds (models 2.2 and 3.3 in Table 5.1). Statistical summaries of the best models are given in Table A5.1.



**Figure 5.4 Patch residence time of treatment birds compared to the reference birds.** Panels A and C show patch residence time as a function of gizzard mass *at release*, and panels B and D show them as a function of gizzard mass *at catch*. Each dot is the mean value of one bird. Dotted lines show model predictions for the reference birds (model 1.1 in Table 5.1, see lines in Fig. 5.3). Solid lines show the treatment birds, where the slope is set equal to the dotted line (models 2.1 and 3.1 in Table 5.1, in which model 1.1 is used as offset). As shown by the vertical distance between the regression lines, the observed patch residence times match better with their gizzard mass *at catch* (panel B and D, models 2 in Table 5.1) than with their gizzard mass *at release* (panel A and C, models 3 in Table 5.1). Note that the axes are log-scaled.



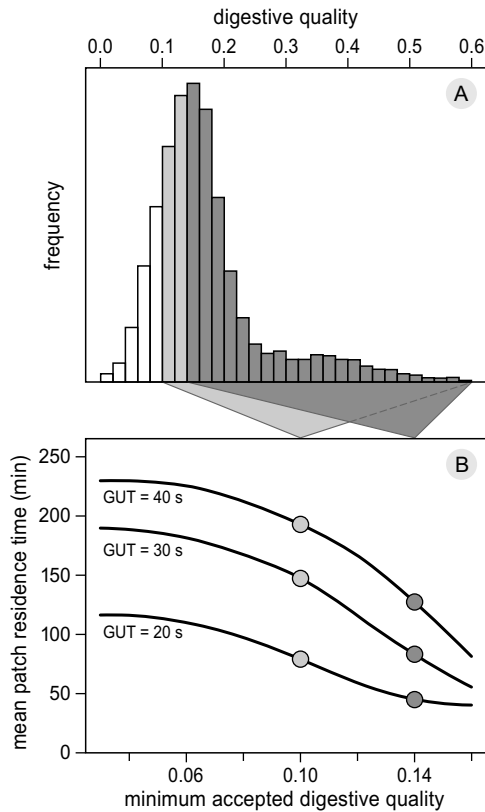
**Figure 5.5 The effect of day since release on patch residence times.** Shown are the residual values of model 1.1 (Table 5.1) against the day since release of the reference birds (A) and the treatment birds (B). The predicted log-transformed patch residence times are back-transformed before subtraction from the observed values. Open grey dots show the mean values per low tide averaged per bird. Black dots show the mean of those values per day since release. Black lines show LOESS regressions (span = 0.7) on the model residuals.

### Temporal trend in patch residence times of treatment birds

There was a tendency for increasing patch residence times in the treatment birds in the first week after release (Fig. 5.5). However, restricting the data to only the first two days after release, when mean patch residence times by the treatment birds appeared to be lower, did not change the qualitative outcome of the analysis. Even then, patch residence times remained longer than predicted from gizzard mass at release (on average 18 min, model A2.1 in Table A5.3, Fig. A5.2), and were as expected from gizzard mass at catch (on average 1 min longer, model A1.1 in Table A5.3, Fig. A5.2). Hence, although patch residence times were lower in the first days after release than thereafter, the treatment birds did not show lower patch residence times than expected from their original gizzard mass.

### Explaining patch residence times from diet preferences

At the 44 identified feeding locations, average available and ingestible prey densities were 1104 molluscs  $m^{-2}$ , ranging from 0 to 9394  $m^{-2}$  (SD = 1644). The frequency distribution of their digestive quality is given in Fig. 5.6A. The average giving-up time of free-living radio-tagged birds in the wild was 33 s, ranging between 3 s and 245 s. Based on the densities and prey quality distribution at the individual locations, simulations predicted that patch residence time should decrease when the minimum acceptable prey quality is increased (Fig. 5.6B). Repeating the simulations with giving-up times of 20, 30 and 40 s showed that expected patch residence time increases with giving-up time. Independent of the used giving-up time, the expected patch residence time decreased considerably with an increase in the minimum acceptable prey quality (Fig 5.6B). The range of expected patch



**Figure 5.6 Proof of principle: simulating the effect of prey preferences on patch residence times using measured prey abundances.** (A) Given the observed densities and frequency distribution of prey qualities, a forager that accepts all prey above a quality of, for example, 0.14 (dark grey bars) perceives a lower prey abundance than an animal that accepts all prey above a lower quality of, for example, 0.10 (light and dark grey bars combined). (B) When assuming that all animals have a fixed searching efficiency and giving-up time (GUT), the animal that accepts all prey above a quality of 0.10 is expected to depart later (light grey symbols vs. dark grey symbols). Simulations for three different giving-up time values are shown. Average measured giving-up time was 33 s.

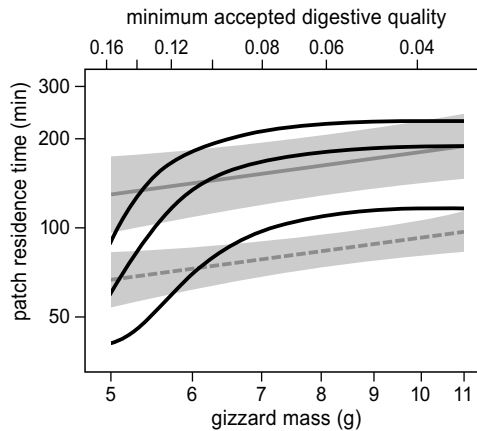
residence times from the statistical models and the simulations are of the same order of magnitude, as are the estimated effect sizes of gizzard mass (Fig. 5.7).

## DISCUSSION

The results of this study validate the proposed positive correlation between gizzard mass and patch residence time in free-roaming red knots (Bijleveld *et al.* 2016, Fig. 5.3), and show that individual diet preferences can indeed explain the observed among-individual variation in gizzard mass and patch residence time (Fig. 5.7). In agreement with an earlier test in captive red knots, a reduction in gizzard mass did not cause an adjustment in patch residence times of free-roaming red knots (Fig. 5.4A and C). These results are consistent with the suggestion that gizzard mass variation is the consequence rather than the cause of behavioural differences in red knots (Bijleveld *et al.* 2014).

### Individual differences in giving-up time

Consistent differences in parameters such as searching efficiency and giving-up time may explain part of the large residual variation in a mean patch residence times (Fig 5.3, Table A5.1). Fig. 5.7 shows that differences in giving-up time are expected to have a large effect on patch residence time. Indeed, variation in the observed giving-up times was high, and hence, these field measurements should be regarded as an indication of the order-of-magnitude rather than a precise estimate. In reality, giving-up time is expected to differ



**Figure 5.7 Predictions of the giving-up time model, compared to observed patch residence times.** The black lines show simulated patch residence times as a function of gizzard mass for three giving-up time values (20, 30 and 40 s, from lower to upper line), assuming that birds maintain the gizzard capacity that is needed to fulfil energy demands on prey of the minimum accepted quality (upper x-axis). The thick grey lines show the loglinear regressions of the observed patch residence time against gizzard mass in the reference birds during the night (solid) and in daytime (dotted) (see Fig. 5.3). The 95% confidence intervals of the regressions are shown by the grey areas (only the uncertainty in the fixed effects of the regression model is considered). Note that the lower x-axis and the y-axis are log-scaled.

between individuals that differ in diet preferences and hence perceive a different food distribution, since giving-up time should depend on the expected prey encounter rate in the patch relative to the expected encounter rate in other patches (McNair 1982; Green 1984; van Gils *et al.* 2003b). This also may explain why the simulated values actually extended beyond the 95% confidence-interval of the mean observed patch residence times as a function of gizzard mass and time-of-day (Fig. 5.7). Birds with very small gizzards may in reality have higher giving-up times because their expected encounter rate may be lower in general.

### **Treatment effect**

Although patch residence times were lower in the first days after the release than thereafter (Fig. 5.5), the treatment birds did not show lower patch residence times than expected from their original gizzard mass (Fig. A5.2). Contrarily, after a few days in the field, the treatment birds started showing a tendency for even longer patch residence times than expected from their original gizzard mass (Fig. 5.4B and D). This may have resulted from other potential effects of the treatment, including e.g. stress or feather damage. Furthermore, the temporary absence from the field in itself may have had short-term effects on mean patch residence times, for example, by influencing up-to-date information on the environment and social status among conspecifics.

### **Differences between night and daytime**

Interestingly, patch residence times at night were longer than in daytime (Fig. 5.3). Because mollusc prey are sessile and are found by touch rather than by sight (Piersma *et al.* 1995), this is unlikely caused by day-night differences in searching efficiencies. Instead, we propose that the longer patch visits during the night were a consequence of predation avoidance behaviour. Predation risk is a factor known to influence habitat selection in general (Lima & Dill 1990), and is known to influence the spatial distribution of red knots at Banc d'Arguin (van den Hout, Spaans & Piersma 2008; van den Hout *et al.* 2014). Falcons and harriers are mainly active during the day, whereas owls are active mainly during the night (Bijlsma 1990; van den Hout *et al.* 2014). Differences in the effectiveness of escape behaviours may lead to longer patch residence times at night (Sitters *et al.* 2001; Gillings *et al.* 2005). Sitting still as a defence may be common in the night (Mouritsen 1992), whereas evasion by way of flocking flights (see van den Hout *et al.* 2009) may be more common during the day (Gillings *et al.* 2005; Conklin & Colwell 2007). Although the relative contribution of these effects remains to be studied, antipredation behaviour is likely to have had considerable influence on the observed foraging movements (Bijleveld *et al.* 2014).

### **The ontogenetic development of individual variation**

Since many behavioural traits are known to be influenced by experience, and diet preferences in particular (e.g. Gillingham & Bunnell 1989; Whiteside, Sage & Madden 2015), the results of this study highlight the potential cascading effect of experiences on individual trait expression in general, behavioural as well as physiomorphic. Because individual



differences in trait expression are essential to take into account when estimating ecological dynamics on the population level (Araújo, Bolnick & Layman 2011; Bolnick *et al.* 2011), it is of importance to know the extent to which experience drives the expression of different traits, behavioural and physiomorphic alike. To this end, research on the ontogenetic development of traits and their consistency over the animal's lifetime is highly relevant (Stamps & Groothuis 2010a). For example, the influence of the environment on expression may decrease with age in some traits, but not in others (Senner, Conklin & Piersma 2015). Comparing these developmental effects between different populations may provide knowledge on the conditions that determine them. These may include, for example, the amount of spatial or temporal heterogeneity in prey density, prey quality and predation risk (as proposed by e.g. Gabriel *et al.* 2005; Mathot *et al.* 2012).

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### DATA ACCESSIBILITY

Data is available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.2s8rr> (Oudman *et al.* 2016)

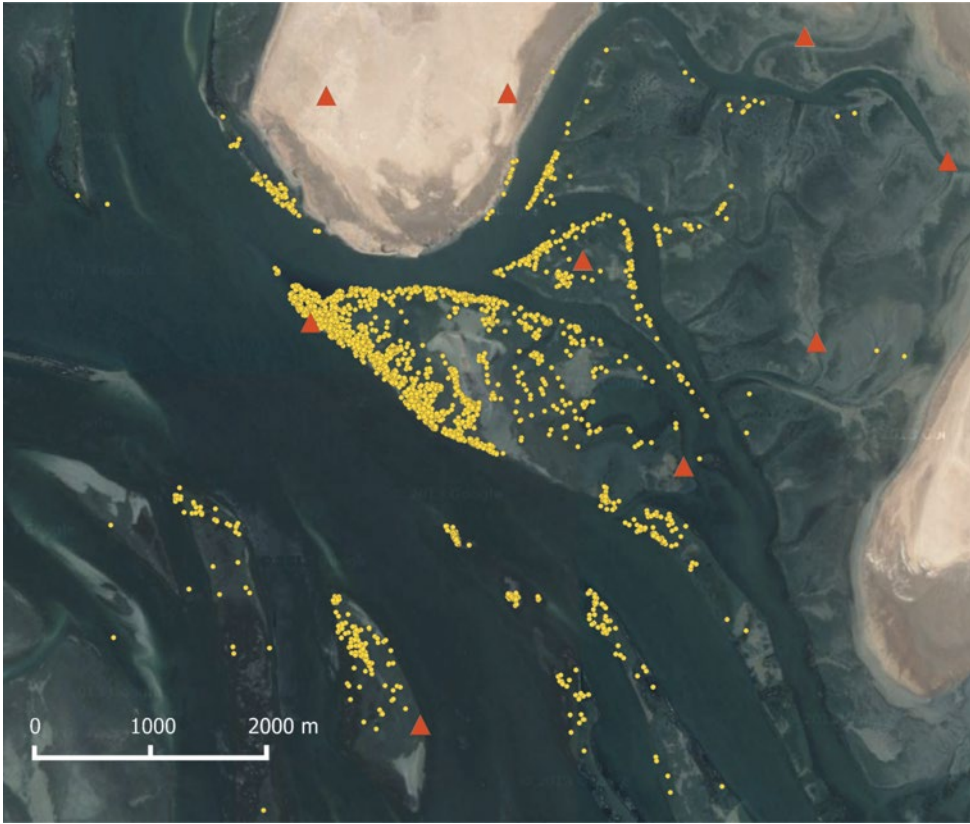
## APPENDIX 5.1. Simulating patch residence times

Under the assumption that birds differ in the minimum digestive quality of prey that they ingest (Bijleveld *et al.* 2016), we constructed a quantitative simulation model to predict patch residence times of individuals with different digestive capacities (as expressed by gizzard mass). We also assume that the decision to leave a patch is a function of the expected encounter rate of acceptable prey, independently of previous encounters (e.g. van Gils *et al.* 2003b). For the sake of simplicity, we define that a bird will leave a patch when it does not encounter any acceptable prey within a critical period of time (giving-up time; Krebs, Ryan & Charnov 1974). When we further assume that red knots search for their prey randomly (van Gils *et al.* 2003b), the search times between prey encounters should be exponentially distributed. Residence times were estimated by drawing a sequence of search times from the exponential distribution with an average encounter rate of  $ax$ , where  $x$  is the density of acceptable prey (Olsson & Holmgren 1998; van Gils *et al.* 2003b) and  $a$  is searching efficiency ( $4 \times 10^{-4}$  m<sup>2</sup>/s; Piersma *et al.* 1995; de Fouw *et al.* 2016). The sequence ended when a search time was drawn that surpassed the giving-up time. Residence time was calculated by adding all search times and handling times (1 s per prey; de Fouw *et al.* 2016), with a maximum of 6 hours (the length of one low tide period).

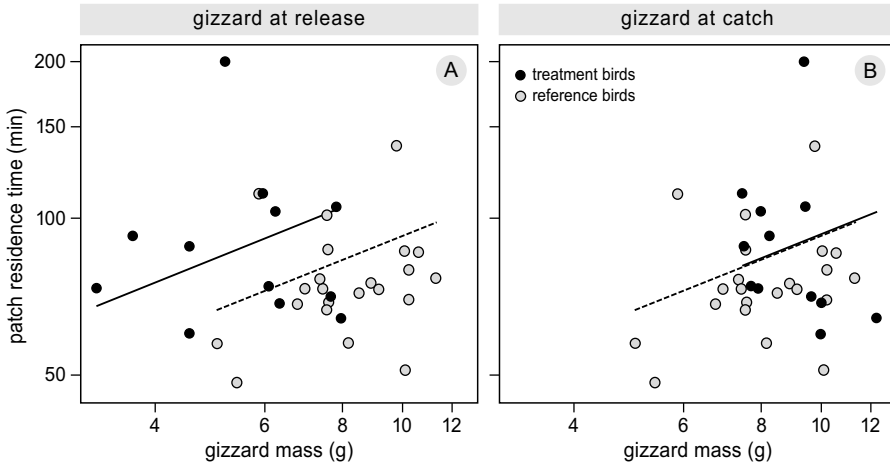
Prey densities and prey qualities were estimated at 44 locations where foraging tagged red knots were visually observed, as described in the main text. The density of acceptable prey (individuals/m<sup>2</sup>) was estimated by dividing the total number of prey items with a predicted digestive quality above the minimum acceptable quality by the sampled surface area. The simulation was repeated 100 times for each observed prey density and the corresponding distribution of prey qualities, making a total of 4400 simulations. The model predictions in figures 5 and 6 were compiled by repeating these simulations for 27 different values of minimum accepted prey quality, ranging from 0.03 to 0.16.

Giving-up time was estimated by video-recording the observed tagged red knots. In 12 cases we registered a prey ingestion and a subsequent departure event. Time between the last prey capture and patch departure was on average 33 s, with a large standard error (SE = 18 s). Therefore, the procedure was repeated with different giving-up times (Fig. 5.5 in the main text; 20, 30 and 40 s). This shows that the absolute expected patch residence times were largely dependent on the giving-up time estimate. Nonetheless, the predicted change with diet preferences was similar (see Fig. 5.5 in the main text).

The relationship between diet preferences and gizzard mass was estimated by assuming that birds maintained the gizzard mass necessary to meet the required energy intake (appr. 0.2 mg ash-free dry flesh per second in Mauritania, van Gils *et al.* 2009) on prey of the least acceptable quality. Shell mass processing capacity has been shown to be a quadratic function of gizzard mass,  $IR = 10^{-1.293}G^2$ , where  $IR$  is the intake rate of dry shell mass (mg/s) and  $G$  is the gizzard mass (g) (van Gils *et al.* 2003a; Chapter 4).



**Figure A5.1 Map of all residence patches.** Shown is an image of the research area, and the positions of all residence patches that were used in the analysis, marked as yellow dots. The islet of Zira is in the middle. Positions of the nine radio receiver stations are marked by orange triangles.



**Figure A5.2 Patch residence times of the treatment birds during daytime on the first two days after release.** Panels A and B are equivalent to Figure 5.3A and B respectively, except for that the data is restricted to the first two days after release of the treatment birds. During these two days, there was no tide during the night. Panel a shows patch residence time as a function of gizzard mass at release, panel b shows them as a function of gizzard mass at catch. Each dot is the mean value of one bird. Dashed lines show the statistical model predictions for the reference birds (model 1.1 in Table 5.1). Solid lines show the estimated intercept of the treatment birds, where the slope is set equal to the dashed line (models A1.2 and A2.1 in Table A5.2, in which model 1.1 is used as offset). Note that the axes are log-scaled.

**Table A5.1 Parameter estimates of the best statistical models.**

	Effects	Offset	Estimate	SE	t-value
Model 1.1	Intercept		1.49	0.16	9.36
	Gizzard	0.48	0.17	2.79	
	Night		0.29	0.06	5.13
	(1 Bird)		0.05		
	(1 Tide)		0.12		
	Residual		0.26		
Model 2.1		$1.49 + 0.48 * \text{Giz}^{\text{catch}} + 0.29 * \text{Night}$			
	Treatment		0.09	0.03	2.7
	(1 Bird)		0.09		
	(1 Tide)		0.14		
	Residual		0.26		
Model 3.1		$1.49 + 0.48 * \text{Giz}^{\text{release}} + 0.29 * \text{Night}$			
	Treatment		0.19	0.04	5.4
	(1 Bird)		0.10		
	(1 Tide)		0.14		
	Residual		0.26		

NB: All models are linear mixed-effects models (function 'lmer' in package 'lme4'), with  $\log_{10}(\text{patch residence time})$  as response variable, measured in minutes. Parameters were estimated by maximizing the log-likelihood. Gizzard masses were measured in g and  $\log_{10}$ -transformed.  $\text{Giz}^{\text{catch}}$  in the offset of model 2.1 refers to gizzard mass at catch,  $\text{Giz}^{\text{release}}$  in the offset of model 3.1 refers to gizzard mass at release. (1|Bird) and (1|Tide) refer to the random variables Bird-ID and Tide-ID. Estimates of the random variables and the residuals refer to standard deviations from the fixed estimates.

**Table A5.2 AICc comparison of statistical models, using only data from the first two days after release of the treatment birds.**

Model	Fixed effects <sup>a</sup>	K <sup>b</sup>	$\Delta\text{AICc}$	AICc weight	Cum. weight	LL <sup>c</sup>
<b>A1.1</b>	<b>Offset(model 1.1, giz at catch)</b>	<b>3</b>	–	<b>0.68</b>	<b>0.68</b>	<b>–3.8</b>
A1.2	Offset(model 1.1, giz at catch) + Treatment	4	2.13	0.24	0.92	–3.8
A1.3	Offset(model 1.1, giz at catch) + Treatment + Diet	5	4.24	0.08	1	–3.8
<b>A2.1</b>	<b>Offset(model 1.1, giz at release) + Treatment</b>	<b>4</b>	–	<b>0.56</b>	<b>0.56</b>	<b>–122.4</b>
A2.2	Offset(model 1.1, giz at release) + Treatment + Diet	5	1.39	0.28	0.84	–122.4
A2.3	Offset(model 1.1, giz at release)	3	2.47	0.16	1	–133.4

NB. Models are linear mixed-effects models (function 'lmer' in package 'lme4' in R), with tide-ID and bird-ID as random intercepts. Best models are in bold (Burnham & Anderson 2002). Parameters were estimated by maximizing the log likelihood. Log-transformed patch residence time is the response variable in all models, which is averaged per bird per low tide after transformation.

<sup>a</sup> Models A1 contain gizzard masses as measured when the birds were caught, models A2 contain gizzard masses before release. Factor 'Treatment' refers to whether the bird was in the treatment group or the reference group. Diet refers to the diet group within the treatment group (either a soft diet or a partially hard-shelled diet).

To test whether the treatment birds deviate from the predictions derived from the reference bird data, the estimated coefficients of the fixed effects of model 1.1 are used as an offset in the models.

<sup>b</sup> The number of parameters in the model.

<sup>c</sup> Log likelihood.

